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GENETIC STUDIES ON THE NATURE OF CANCER[1]

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In 1889 Hanau reported on the successful transplantation of carcinoma from one individual to another of the same species. This experiment did not receive immediate recognition. Gradually, however, such early experimental pathologists as Jensen, Leo Loeb and Borrel initiated a movement which had as its aim the biological investigation of the cancer problem. This movement was apparently started by a communication by Jensen in 1903.

Simultaneously, some of these investigators and many others were studying the occurrence of spontaneous neoplasms in various animals. Since the methods, the results and probable significance of the data of these two general fields of research are quite distinct, it may be well to keep the two lines separate. It is necessary to bear in mind that the origin of the neoplastic cell is quite distinct from the progressive growth of the same cell even within the same host. Although from the biological viewpoint the transplanted cell must have some characteristics in common with the spontaneous cell, yet in the process of transplantation certain complicating factors are introduced. The spontaneous cell is directly descended from cells derived from normal tissue cells of

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the individual that gave rise to the neoplasm. This relation between host tissue and neoplastic tissue does not exist in the case of the transplanted tissue, since in this case the neoplastic cells are indirectly descended from the original individual that gave rise to the tissue (in the first place). It is also assumed that the fact that transplantable tumors often show a large percentage of retrogressive masses differentiates this tissue from spontaneous tissue, in which there is practically no spontaneous regression.

We will consider first the work on the origin of spontaneous growths. We desire to point out only those observations that have a possible bearing upon the genetic viewpoint.

I

THE SPONTANEOUS TUMOR

In 1899, Loeb, working with Jobson, made some observations on the endemic occurrence of cancer in the stockyards of Chicago. He noted especially that cattle coming from a certain ranch in Wyoming were particularly susceptible to cancer of the inner canthus of the eye. Later, Loeb observed a large incidence of sarcoma of the thyroid in a family of rats. He pointed out the probability that the occurrence of these peculiar neoplastic growths may be due to a hereditary condition rather than to an infection. He then started quite an extensive investigation in conjunction with Miss Lathrop, of Granby, Massachusetts. It was soon apparent that cancer occurred with much greater frequency in certain inbred strains than in others, and also that no indications whatever could be found of cage infection or direct infection from animal to animal. Their preliminary conclusion (1907) was that there existed a hereditary predisposition which was responsible for the endemic occurrence of cancer. I shall refer to the more detailed analysis of their work later.

The probable bearing of heredity on the incidence of cancer early attracted other investigators. E. E. Tyzzer

(1907) concluded that heredity may play a part in the etiology of animal cancer. Simultaneously, Bashford and Murray (1909) and later Murray (1911–12) undertook similar studies, using the same statistical methods that had been employed in the study of human cancer. Murray compared the incidence of cancer among individuals whose direct ancestry showed cancer on the one hand, and among those in whose ancestry cancer had not occurred for at least three generations back. The incidence of cancer in individuals whose ancestry had shown cancer was about twice as high as in those individuals in which cancer had not occurred in the preceding two generations.

These early results, together with those of Albrecht, Hecht, Spronk and a few others, indicated a probably significant bearing of heredity on the occurrence of cancer.

At this time, however, there were alternative views, such as those of Borrel and others who referred the endemic occurrence of cancer not to heredity but to infection, and as late as 1910 Bashford expressed the opinion that heredity has no significance in the causation of cancer. The conception that cancer is an infectious process and, therefore, the endemic occurrence of cancer may be comparable to an "epidemic of disease germs" has however been relegated to the background of speculation.

The influence of many pathologists, headed especially by Bashford, has hampered the application of these data on the inheritance of cancer in the lower organisms to similar material in man. This has been unfortunate. I believe this has come about largely by a misconception of the fundamental laws of heredity. Let me quote from a recent text-book on tumors, published in 1920.

It is possible, also in certain families, where the inbreeding has been very close that a general cancer liability may be produced; but that such liability exists to any degree in the whole population has been negatived by the investigations of the insurance actuaries by whom it has been shown that a history of cancer in the ancestry of the insured makes that person no more susceptible to cancer than any other person in the

normal population exposed to the disease. On the other hand, the experiments of Murray have shown that the introduction of breeding mice of cancerous ancestry into certain strains of these animals increased the number of spontaneous tumors appearing in the succeeding generations, the increased rate of occurrence being double the rate of occurrence in the offspring of normal mice. Such results can not, however, be applied to man, as the experimentally produced concentration of cancerous ancestry is greater than could ever occur in the human race. Another point to be considered is—that if cancer is hereditary, the entire race should have been destroyed, because as the disease afflicts chiefly persons after the reproductive age has been passed, it does not interfere with the increase in the population; and, hence, there should have been an increasingly greater concentration of cancer ancestry through the ages, with the result that the disease should be very much more abundant than it now is. Such concentration does not appear statistically in any known race, even though the inbreeding is fairly close so that the original stock is not much diluted by immigration.

These arguments against the acceptance of the hereditary transmission of cancer in man are commonly used. That cancer should be more prevalent than it actually is, granted that it be hereditary, does not follow. One might argue that cancer is probably a very recent acquisition in man and the time element has not been sufficient for the depletion of the human species. Again, this argument may just as well be applied to such post-reproductive hereditary diseases as Huntington's chorea—or any other debilitating hereditary disease. It may also be well to point out certain of the limitations of the use of the term inbreeding. A good many investigators have recently used this term in its obsolete sense, that is, they maintain that the process of inbreeding may introduce something into the stock that would not otherwise arise. That inbreeding merely fixes or brings to the surface something that has been always latent in the germ plasm is a point not yet fully appreciated nor accepted by these pathologists. It is unfortunate that these genetic terms have been misused.

Even if cancer is not hereditary in man, it certainly is in some of the mammals, especially among the rodents, and this problem is of sufficient interest to biologists at this time.

In the past eighteen years a prodigious amount of work has been done on this problem, especially by Loeb, Maud

Slye and more recently by Lynch. It is needless to go through the development of the various conclusions. The fact of inheritance of cancer in the lower organisms can not be disputed. Since, however, there is a divergence of opinion as to the mechanism of inheritance, it may be well to compare these conclusions.

Loeb, with the assistance of Miss Lathrop, started his experiments on a large scale in 1910. His results were obtained on about twelve thousand female mice which had been observed throughout their entire life span. He confined his observations to carcinoma of the mammary gland, the most frequent neoplastic tissue encountered in mice. His conclusions may be briefly summarized:

(1) The cancer-rate of each strain or family is a definite characteristic of this strain and is transmitted by heredity to successive generations. This cancer-rate may vary between zero in certain strains and almost 100 per cent. in others.

(2) These differences in cancer-rate persist with a surprising regularity through successive generations in the majority of strains. In certain strains, however, variations in the tumor-rate do occur. This change usually results in a decreased incidence—due probably to two causes; (a) long-continued inbreeding results in a gradual decrease in fertility and vigor (associated with this is a decreased tumor-rate), and (b) various factors cause a selection to take place within the strains (certain families die out, while others gain preponderance). This results in a changed tumor-rate.

(3) If we cross strains with a similar tumor-rate, the offspring inherit the tumor-rate common to both parents; if both parents differ in tumor-rates, the tumor-rate of the offspring is on the whole intermediate between those of the parents. All degrees of intermediacy are observed. In some cases, the higher tumor-rate dominates over the lower tumor-rate.

(4) The age at which tumors appear is just as characteristic of individual strains as the tumor-rate. The tumor-age is also inherited.

(5) The hereditary tendency to cancer is not a simple quantity but a composite, because (a) the hereditary predisposition to cancer is due to the cooperation of multiple factors and (b) there is also involved a second factor, which again is variable, namely, the activity of the ovary.

(6) In all cases the realization of the hereditary tendency to cancer presupposes the activity of the internal secretion of the ovary. Thus it has become possible to express in a quantitative way the tendency to cancer as due to the interaction of two main factors, both internal, the one hereditarily fixed and the other accessible to experimental variation. Both factors combined are the predisposition as well as the cause of cancer. In the case of other kinds of cancer, conditions are presumably similar—the rôle of the ovarian function, being probably taken over in such cases by other glands with internal secretion or by external stimulation.

(7) The predisposition to cancer is inherited independently of such characters as vigor, prolificity, size and coat color.

(8) External stimuli, either of a chemical or mechanical nature, may also play a part in the incidence of cancer.

Loeb's conception as to the cause of cancer is somewhat as follows: there are two sets of factors involved, one internal, the other external. There is a play between these forces, and as a result of this reaction a neoplastic tissue results. Sometimes, as in the cases where there is obviously a clear-cut source of chemical or mechanical stimulation such as coal tar, or the presence of an organism such as Fibiger's Spiroptera, the external forces predominate; at other times, when there is apparently no discernible external stimulation, then the internal factors, which are inherited, play the leading rôle. In every case, however, there is the activity of some internal secretion—from the ovary in the case of the mammary gland tumors.

A few criticisms on this work may be pointed out.

(a) Granted that cancer is due to the activity or coöperation of multiple Mendelian factors, since Loeb found that the tumor-rate varied in different strains from zero to 100 per cent., it is more than probable that he was not dealing with homogeneous material. Consequently, his results on hybridization are probably not valid, although very suggestive.

(b) There apparently was no consistent employment of the use of selection in the establishment of homogeneous material—merely the tabulation of data as they accumulated in the laboratory.

(c) The data in many cases are too meager and inconclusive.

(d) That a decrease in the tumor-rate may be due to a loss of fertility and vigor by long-continued inbreeding is not borne out by the facts. It has been demonstrated that inbreeding as such does not necessarily result in a decrease in these physiological characteristics. On the other hand, T. B. Robertson has recently expressed the opinion that the more vigorous individuals, determined by the developmental rate, are more prone to become cancerous.

The data do, however, prove the inheritance of susceptibility to neoplastic tissue. Multiple factors are also apparently indicated in several cases.

A little later, Miss Maud Slye became interested in the probable inheritance of cancer and for the past fifteen years has done an enormous amount of work on this problem. In a general way, her conclusions are in agreement with Loeb's. She has continued the work into several divergent fields. In certain particulars, her conclusions differ considerably from Loeb's. The reader is referred to a recent article by Miss Slye in the April, 1922, issue of the *Journal of Cancer Research,* from which article this brief discussion has been obtained. In this paper, one is struck by the use of a syllogism which may be stated as follows:

(a) All efficient study of heredity is the study of the behavior of unit characters.

(b) Cancer is hereditary. (She has demonstrated this fact on observations of more than forty thousand mice).

Therefore,

(c) Cancer is a unit character, and is the result of the activity of a simple unit factor. Furthermore, this simple unit factor is recessive to the factor that results in the condition of non-cancer.

Her conclusions are briefly:

(1) Cancer and non-cancer tendencies segregate out and are transmitted as such.

(2) They are therefore unit characters.

(3) A specificity of tissue type in specific organs from ancestor to offspring segregates out and is transmitted as such.

(4) It is therefore a unit character.

(5) Since these things are unit characters, it is possible to manipulate them by selective breeding and thereby to implant them indelibly in any species or to eliminate them permanently and completely from any species.

(6) Cancer and non-cancer behave like the absence and presence, respectively, of a mechanism fitted to control proliferation and differentiation in regenerative processes.

There is a large mass of data that indicate that the various types of cancer both as to histological appearance and situation within the organism are unit characters since they segregate out and retain an individuality

in successive generations. It does not follow, however, that these "unit characters" are produced by unit factors. It is well to keep in mind also that the number of genetic factors segregating out in any hybridization experiment depends upon the genetic constitution of the individuals crossed and does not indicate the absolute number of genetic determiners involved in the production of the characteristic.

It is seen that Miss Slye's interpretation of the inheritance of cancer susceptibility differs from Loeb's conception in two main details. (1) Miss Slye attributes everything to heredity. On the other hand, Loeb believes that cancer is produced by secondary extrinsic or environmental forces working upon an internal or genetic mechanism. He does not point out, however, that the second part of his intrinsic factor, namely, the activity of the ovary, or of internal secretion in general, may also, in the last analysis, be controlled by genetic determiners. (2) In the second place, Miss Slye believes that she has definite evidence that cancer is due to a single recessive factor. Loeb's data seem to necessitate the assumption of the activity of multiple factors.

Quite recently, Miss Lynch, of the Rockefeller Institute, has reported on the relation existing between tumor susceptibility and heredity. She has introduced several refinements in method for a proper genetic interpretation of the data obtained. She employed not only the F_2 generation produced by originally crossing two strains of animals that differed significantly in their tumor incidence but also the back-cross generation. In this first report she points out that the frequency with which tumors appear in the first filial generation indicates that the character is apparently dominant, in contradiction to the interpretation reported by Miss Slye. She also points out the inadequacy of Slye's material for proper analysis.

In concluding this survey of the genetic interpretation for the incidence of endemic occurrence of tumors in laboratory stocks of mice, I shall add a few general

remarks. In the first place, it is quite evident that cancer is inherited. Furthermore, the mechanism of inheritance has not been determined with any degree of certainty. There is strong presumptive evidence that unit factors (probably multiple) are functional in the incidence of cancer. Whether these factors are "all sufficient" for the production of cancer or whether they produce merely a substratum on which certain extrinsic factors, such as irritation, must act remains to be demonstrated. It has been demonstrated, however, that certain chemical substances, such as coal tar, are apparently potent enough to completely wipe out the inherited tendency of the individual experimented upon. At the present time, it is extremely difficult to determine the relative importance of these two complexes in the incidence of cancer. Perhaps this is an obstacle in the path of those who are trying to approach the cancer problem from the standpoint of the inheritance of susceptibility to spontaneous tumors. There is also another drawback to the use of this method exclusively. None of this work visualizes the actual production of the neoplastic change—that is, how does the normal tissue change over into neoplastic tissue? The work assumes, and it has been maintained without actual evidence that mutations or genetic deviations lie at the background of these changes—but this mutational process has not been demonstrated nor proven by this procedure, nor is it likely to be so demonstrated. The recent work of Bagg on the hyperactivity of the mammary gland also suggests a complicating factor not fully accounted for on this earlier work on the incidence of spontaneous cancer.

So much for the inheritance of cancer in animals. There has been some work that demonstrates the inheritance of cancer in humans. This is especially true of such tumors as retinal glioma and fibroma molluscum.

The study of so-called "cancer families" has not always been fortunate.

In the first place, the earlier pedigrees did not always include all the non-cancerous relatives. Secondly, chance

alone would account for the occurrence of several cancers in a single family. Thirdly, the tabulation of the data has been obtained either from hearsay evidence or the inexpert knowledge of laymen.

The Bonaparte family is frequently noted for the large incidence of cancer of the stomach; Napoleon I, his father, his brother Lucien, and two of his sisters, Pauline and Caroline, all having been reported as dying of this particular malady. The family of Madame Z is another of similar kind. The strongest case for the conclusion that certain hereditary tendencies to cancer are evident is furnished by those cases in which a peculiarly rare or peculiarly situated tumor appears in a large number of individuals belonging to the same family. There have been reported a large number of cases of retinal glioma appearing in near relatives. Families showing this rare tumor have been reported by Newton (1902), Wilson (1871–74), Purtscher (1922) and Comas (1920). Other families showing the occurrence of other extremely rare tumors have been recently reported by Silcock (1892), Williams (1909), Bashford (1909), Pel (1915), Peiser (1915), Watkins (1904), Richards (1921), Oidtman (1917), Hedinger (1915) and Primrose (1920).

At the present time, when so much interest is turned toward the problem of twins, it may be well to cite two remarkable cases that must be beyond random distribution of cancer. Buckard (1922) reported that twin sisters, aged twenty-one years, both developed a fibroadenoma of the left breast, each of the same microscopic structure, at almost the same time and in the same part of the breast. Critzman cited the case of the family in which all the individuals who were not twins died of cancer, whereas all those who were twins escaped. Wolff (1911) maintained that from 11 to 18 per cent. of cancer patients show a history of the malady in the immediate family. Several other neoplastic conditions, strikingly rare as to occurrence in the general population, are very frequent in near relatives. These conditions are sometimes associated with other developmental anomalies and

indicate a strong probability of an inherited susceptibility. Among these may be mentioned multiple benign epithelioma, multiple cartilaginous exostosis and Van Recklinghausen's disease or multiple neurofibromatosis. Davenport indicated that the tendency to neurofibromatosis is hereditarily transmitted as a dominant. More recently Little demonstrated biometrically that the incidence of cancer was significantly higher in the offspring from cancerous parents than was to be expected from random distribution in the general population. Garman (1913) pointed out that the tendency towards cancer differed considerably in the various strains and races of mankind.

Warthin (1925) has published further notes on his so-called cancer family. He noted the occurrence of twenty-eight cases of carcinoma out of a total number of 146 individuals or 31.81 per cent. cancer. And from these data (obtained by lumping together the total data from six successive generations) he concludes that susceptibility is due to a single recessive factor. On the other hand, if one analyzes the material submitted, it is readily apparent that these data may be explained genetically, if one assumes that carcinoma is produced by a single dominant factor. Expectation in nearly every case is very closely met.

For a review of the literature on the inheritance of cancer, the reader is referred to an article by H. Gideon Wells, "The Influence of Heredity on the Occurrence of Cancer," in the September 22, 1923, issue of the *Journal* of the American Medical Association.

II

The Transplantable Tumor

The first investigator who pointed out that heredity played a part in the incidence and development of transplantable tumors in experimental animals appears to have been Morau (1892–94). Several investigators had previously indicated that neoplastic tissue could be transplanted. Among these may be mentioned (1) Hanau

(1889), who succeeded in transferring a carcinoma from one rat to two others, (2) v. Eiselsberg (1890), who produced a transplantable nodule in one rat out of two inoculated, and (3) Firket, who had transferred a sarcoma from one rat to other individuals through four transfer generations. It was shortly after this time that the process of transplantation was put on an experimental basis, largely through the efforts of Jensen, Loeb and Borrel. It was soon apparent that not all transplantable tissues behaved alike. For example, of the one hundred and eight neoplastic tissues employed by Ehrlich (1907) only about 8 per cent. proved to be capable of transplantation into other individuals. Again, only a small percentage of the inoculated individuals would grow the transplants progressively—10 per cent. of "takes" being considered a successful experiment. Loeb (1902) determined that, when two neoplastic tissues derived from the same mass were inoculated simultaneously into a series of rats, either both of the transplants grew or else both failed to develop. This indicated that the individuals employed introduced a variable factor other than the one produced by the transplants themselves. From such a beginning, the problem of the relative influence of host and graft was taken up, especially by the English investigators, Bashford, Murray and Cramer (1905). Their experiment bearing upon this problem consisted in the inoculation of two series of mice with grafts derived from the same source. In one series, the mice were inoculated with a single graft; in the second series, the individuals were inoculated with five grafts of the same tissue. It was found that the series with multiple inoculations of the same tissue produced a higher percentage of progressively growing tumors than the one in which the mice received only single transplants. It was concluded that the influence of the host was very unimportant compared to the influence of the transplantable tissue. Several other experiments were performed, each being interpreted as indicating a minor importance of the stock of mice employed.

Jensen (1903) determined that there was a natural difference of susceptibility between various races or strains of domestic mice. He determined that at first the transplantable tumor was highly specific, that is, that it grew well only in a few individuals belonging to the original stock of mice from which the transplant had been obtained. By inoculating a tumor (that had been obtained in an albino race) into the common gray mouse he found that only one gray was susceptible out of ten used. Soon, however, an increasing number of successful takes was obtained until finally twenty-seven out of eighty-four inoculated grew the transplant progressively, although at a somewhat slower rate. Jensen tried to transplant this tumor into other species of mice but without success. He also determined that various strains of rats differed in their innate tolerance or resistance to the same rat tumor. He determined that an increased percentage of successfully growing rat tumors could be obtained by continued transplantation.

Loeb had pointed out in 1901 that race probably played a rôle in the process of transplantation. In 1905 Loeb reported an experiment that has played quite a part in the development of the present problem of the transplantable tumor. He had determined that a tumor arising in a Japanese Waltzing mouse would not grow progressively in individuals belonging to the common house mouse variety.

The problem of the determination of the suitability of foreign stocks of mice to transplantable tumors received considerable attention from Bashford, Murray and Cramer. Jensen's tumor, having originated in mice obtained from Germany, had proven to be difficult of transplantation into Danish mice. The English investigators tested the transplantability into various stocks of English origin. They completely verified Jensen's original observations. It was evident that only a slight difference in the stock of mice employed introduced complicating factors. However, after a considerable time, a progressively increasing number of successfully growing

tumors could be obtained. The fact that Jensen's tumor had not changed in its capacity to grow in German mice was proven by reinoculating it into these mice after it had been kept in strains of English origin for several generations. Jensen also verified these findings in the case of rat tumors.

That race had anything to do with the observed data was flatly denied by Hertwig and Pol (1907). They pointed out that the common white mouse had been an article of commerce for several years, fifty to sixty thousand being exported from Berlin alone each year. Consequently, an investigator could not be sure of the origin of his material. They strenuously opposed the view that mouse cancer was transferable only between near relatives. They also believed that differences in breed or in diet did not constitute any difficulty for successful transplantation. Haaland (1905) determined that Jensen's tumor would grow successfully in mice found in Norway that had been imported from Germany several years before. Haaland suggested that the tolerance of particular breeds of mice depended largely upon the general conditions under which they had been kept for a long time. Several difficulties of accepting this interpretation were raised, among which Haaland was able to point out some from his own data. He synthetically mixed Ehrlich's sarcoma with Jensen's carcinoma. He then determined that Berlin mice were able to sift out the sarcomatous element of the mixture, whereas in the Danish mice only one pure carcinoma was obtained. Thus was demonstrated a specificity of tissue components that has played such a prominent part in recent developments. Haaland was consequently led to conclude that special and specific factors were involved in the tolerance or resistance to transplantable tumors—not general conditions, as he had originally posited. Later Haaland concluded that diet may have an influence on differential susceptibility. He was led to this conclusion by moving some of his stock from Germany to Norway. He had found that his stock in Germany had been highly sus-

ceptible to Ehrlich's sarcoma, whereas in Norway the descendants of this German stock proved to be highly resistant. This observation should not have much weight, since only six individuals were inoculated.

Observations of this kind increased rapidly. Contradictory results and interpretations were prevalent. There was very little progress. It was at this time that the idea arose that the problem of transplantation would not develop very far.

Several difficulties are evident which at that time had not been taken into consideration: (1) The number of observations of a crucial nature was too small for reliability. (2) "Market mice" or "mice derived from a few dealers only" were employed. This factor is a very important one and not yet fully appreciated. In fact, some leaders have begun to appreciate a careful selection of stocks of mice for genetic analysis. But even with this advance, more caution is necessary. For example, certain investigators refer to "inbred" stocks as those derived by the non-introduction of foreign mice. It has been demonstrated biometrically that this method of mating leads to no further fixation of homogeneous material than does a system of promiscuous matings. (3) No proper hybridization crosses were performed—hence the impossibility of proving or disproving whether the phenomenon of transplantation was even genetic (hereditary) or not. (4) No investigator trained in the science of genetics was interested in this field of research. This of course was to be expected, since the science of genetics was yet in a very immature state of development.

The next important step for the genetic interpretation of transplantation phenomena was taken by Tyzzer in 1909. He confirmed a previous observation of Loeb that ordinary tame mice were quite resistant to a neoplastic tissue that had arisen in a mouse belonging to the Japanese Waltzing strain.

The first tumor of this nature originating in a mouse of the Japanese Waltzing strain was a mammary gland

carcinoma. Individuals belonging to this inbred stock were uniformly susceptible to the transplant. Inoculation into common mice resulted in completely negative results. By crossing these two original stocks of mice together, an F_1 hybrid generation was obtained, all individuals of which were uniformly susceptible. Out of fifty-four individuals raised in the F_2 generation, no susceptible individual was obtained (0 + 54 —). Susceptible individuals could not be obtained in the F_3 generation. Tyzzer concluded from these data that susceptibility to the transplantable carcinoma was inherited, but neither as a mendelian unit character nor in accordance with any other hypothesis of inheritance.

At this time, Little became interested in tumor transplantation work and entered into collaboration with Tyzzer. It was this fortunate combination of an experimental pathologist with a geneticist that paved the way for future advances. Little recognized immediately the necessity of raising a large F_2 generation, since it is in this generation that the crucial test of mendelian segregation was to be obtained. The value of the backcross to the non-susceptible strain had not yet been fully demonstrated. Finally three susceptible individuals out of 183 mice tested in the F_2 generation grew the transplant progressively. Little and Tyzzer (1916) concluded that the data obtained could be explained on the basis of multiple mendelizing factors whose number was estimated at from twelve to fourteen. Simultaneous presence of these factors, themselves introduced by the Japanese Waltzing mice, was considered necessary for the continued growth of the tumor.

Tyzzer then wrote a general paper on tumor immunity in which he surveyed the field of transplantation. He finally arrived at the conclusion through the process of elimination that cancer must be due to the process of mutation. This term had been used several times previously, but always in a descriptive sense, not causal. Of course, the process of mutation may explain the incidence of cancer, but was not by any means proven or

even indicated. With this conclusion, Tyzzer began to realize the futility of ever attempting to prove the process of mutation or developing the work on the genetic interpretation of cancer and gradually took up interests in other fields of research.

Somewhat later, a similar case was investigated by Little, only here a simpler genetic complex was determined. The case involved a transplantable sarcoma. The approximate number of mendelian factors underlying susceptibility was estimated at from three to five, probably four. The work promised to open up a new interpretation for tumor transplantation from the standpoint of genetics. Little, accepting a position at the Carnegie Institution of Washington, took this material along with him. He realized the complexity of the material, but fully appreciated the significance of the conception of multiple factors. In order to facilitate the genetic analysis of the problem, he started the laborious process of inbreeding strains of common mice. It was his foresight that made possible the recent advances in this field. Unfortunately, before a proper analysis of the multiple factor hypothesis could be completed, an epidemic of mouse typhoid completely wiped out the Japanese Waltzing tumors and almost completely the inbred stocks of mice. After this, Little's interests turned to the X-ray experiments, so that practically all the remaining work of a genetic nature has been done by myself.

In 1918 I was studying the reactions of the common wild house mouse to inoculations of neoplastic tissues that had originated in the inbred dilute brown strain. For this purpose I used two mammary gland adenocarcinomata. These tissues were histologically indistinguishable. Yet I discerned a difference in their reaction to the same host tissue. The first tumor was inoculated on the right side of the body, the second on the left side of the same individual. Of course, the fundamental or genetic constitution of the mice must be the same in this comparative work and therefore the difference in the outcome must have been introduced by the tumor tis-

sue itself. I then tested out several stocks of mice for experimental transplantation work. It had been determined some time previously that the inbred dilute brown strain of mice was uniformly susceptible to both transplants. Another inbred strain, known as the Bagg albinos, proved to be uniformly resistant to the same tissues. The F_1 generation, produced by crossing these two ancestral stocks together, gave individuals, all of which were susceptible; that is, all mice grew both transplants progressively and at a rate greater than the original susceptible inbred dilute brown stock individuals. In the F_2 generation the two tumors gave different ratios of susceptible to non-susceptible individuals; the first tumor giving a three factor ratio, the second tumor a two factor ratio.

It was apparent, however, that there was a great similarity between the two tumors. All mice that proved to be susceptible to the first tumor also grew the second tumor, but several mice grew the second tumor that would not grow the first. Some of the individuals were also negative to both tumors. Others grew both. From these data we concluded that for susceptibility to the second tumor there must be involved the simultaneous presence of at least two mendelian factors within the fundamental make-up of the mouse, which have been named A^{st} and B^{st}. The other adeno-carcinoma will grow in a given host, provided there be the simultaneous presence of at least three mendelian units, which have been termed A^{st}, B^{st} and C^{st}. The two tumors are histologically indistinguishable; they possess different physiological activities and they show a differential influence of the host that grows them of one mendelian factor, C^{st}. Since physiological activity of the tumor is apparently independent of morphological or histological structure, the possibility is that it is dependent upon genetic differences. At that time (1924) we expressed the opinion that this genetic difference must be correlated with the single C^{st} factor. This of course does not mean that we believe this genetic difference of necessity lies within a

single chromosome. What we mean by the genetic constitution of the tumor cell is the entire cellular system complex, and any differene or shift anywhere in the system, be it either nuclear or cytoplasmic, would be a change in the genetic or biological constitution. From this experiment, we developed the genetic theory for the transplantation of neoplastic tissue, which may be stated as follows, "the fate of the implanted tumor tissue when placed in a given individual (host) is brought about by a reaction between the host, determined to a large extent by its constitution, and the transplantable tumor cell, controlled to some extent by certain intrinsic factors."

My recent work has been in the testing out of this tentative theory. Since most of the criticisms at first have centered around the main method of attack on the problem, I have deemed it necessary to answer these criticisms before developing the genetic work further. The first criticism that came to my attention was that my results were obtained with special neoplastic tissues and could not be duplicated with other better known transplantable tumors. Tumors have been known that were non-specific, that is, they would grow in all mice irrespective of genetic relationship; consequently the phenomenon of mendelian segregation probably would not apply to them. It was also intimated to me that these non-specific tissues were nearer to human cancers than any others found in mice. To put the problem in terms of genetics, as was done by one of the critics, "it looks as if all the genetic factors necessary to *make mouse* might be sufficient for susceptibility to these non-specific tumors." After inoculating some five hundred individuals, derived from as heterogeneous stocks of mice as I could possibly obtain, I was nearly of this opinion myself, since I had obtained only three negative individuals. Two of these died without leaving any progeny. The other negative individual proved to be a male and he was used quite extensively for breeding purposes. After six years of a very tedious grind, by the use of the selection of negative individuals for breeding purposes, I have

been able to develop a stock one hundred per cent. resistant to this transplant. I have been unable to make any genetic crosses in order to determine more in detail the genetic complex involved in transplantation. With the production of the negative strain, however, I am justified in concluding that "all factors that *make mouse*" do not control the transplantability of this tumor. Specificity and non-specificity are genetic phenomena and must be analyzed by recognized genetic methods. Thus, a case that appeared to be an exception to the rule that specific genetic factors were at the bottom of tissue transplantation has fallen into line with the theory.

The second criticism of my method was that the results were obtained on tumors that had originated in inbred strains and could not be duplicated with neoplastic tissue derived from "market mice." Obviously, some one had an idea that the process of inbreeding had introduced some complicating factors on the production of neoplastic tissue. I, therefore, picked out a tumor that had originated in as heterozygous an individual as I could obtain, an F_1 hybrid between two distinct stocks of mice. It was extremely difficult to get this tumor to grow in heterogeneous individuals. I finally obtained an individual that would grow the transplant progressively. I waited until there was very little prospect of spontaneous regression of the growth setting in, and then operated, removing all the tissue. The individual was then bred to several other mice. Then by inoculating the offspring, I found that some grew the transplant and others did not. I started a selection in both directions, one toward high susceptibility and one toward low susceptibility. Within four years I had developed two distinct stocks of mice, one of which was one hundred per cent. susceptible to the transplant, the other stock one hundred per cent. resistant to the same tissue. Then, by subsequent crosses between the two synthetic strains of mice, I again demonstrated mendelian segregation of specific genetic factors. It is readily apparent that "inbreed-

ing" of pure stocks has clarified the problem of tumor transplantation—not complicated it.

The third point was indicated that "rhythms" of growth were demonstrated. It had been inferred by some investigators that this phenomenon could not be explained by genetics. It may be well to recall that certain of the older investigators had believed that this phenomenon was associated with a rhythm of a life cycle of some hypothetical parasitic organism within the neoplastic tissue. It is also well to bear in mind that this phenomenon had been ascertained on stocks of "market mice." By this time I was of the opinion that transplantation was largely a phenomenon of genetics and consequently I thought that the experiments ought to be repeated on controlled stocks, that is, where pedigrees of all the individuals were carefully kept. This experiment consisted of inoculating a tumor into heterogeneous material (hosts), the pedigrees of all the individuals, however, being known for several generations. Rhythms of a very pronounced character were obtained when all the data for the successive generations were massed. But by analyzing the rhythm curve, it was found that all susceptible individuals were descended from susceptible parents of the preceding generation, and all non-susceptible individuals were descended from non-susceptible parents or parents known to be heterozygous for susceptibility. The rhythm of activity curve could be resolved into two straight or nearly straight lines. At the end of the experiment when both lines were homogeneous and therefore constant, genetic crosses were made. Mendelian segregation of specific susceptibility factors was indicated. Thus could be demonstrated that the so-called "rhythms," as far as this single tumor was concerned, were an artifact and could be artificially controlled by using relative numbers of individuals from the high and the low lines in successive generations. Susceptibility and non-susceptibility were again due to the segregation of definite genetic factors. I do not desire to state, however, that rhythms do not occur in other well-controlled material. All I do wish to point out is that, if rhythms

do actually occur, they ought to be demonstrated on controlled genetic material.

Several years ago, Apolant distinguished between two fundamental characteristics of the neoplastic tissue, (1) transplantability, determined by the number of successfully growing subtransplants, and (2) proliferative energy or vigor, estimated by the rate of growth of the transplant. That transplantability is nothing more nor less than the result of Mendelian segregation of multiple factors within the fundamental make-up of the host harboring the transplant has been fairly well demonstrated. I have been able to accumulate some data bearing upon the problem of the so-called proliferative energy of the neoplastic tissue. It may be recalled that I had demonstrated that F_1 hybrids would grow the transplantable tissue significantly faster than homozygous susceptible mice. Susceptibility had been demonstrated to be one hundred per cent. in both generations. Here was a demonstration of the distinction between these two characteristics of the neoplastic tissue. It occurred to me that a genetic analysis of the rate of growth might be made. (It had been recognized for some time that the rate of growth was quite variable and unpredictable in market mice—fluctuations were always being encountered.) By plotting the growth rates of several hundred susceptible F_2 individuals on one chart, it was indicated that instead of a uniform spread of variability, the individual curves tended to clump—open spaces appearing throughout the chart. When the chart was submitted to several individuals, they were of opinion that four groups were apparent. By the genetic analysis of susceptibility for this tumor, it had been ascertained that there were to be expected four classes of susceptible individuals occurring in this generation (AABB, AaBB, AABb and AaBb). I therefore reached the tentative conclusion that the variations of the growth rate in the various generations of mice were produced by the genetic recombination of these "transplantability" factors. By the accumulation of more data, however, it was apparent that this hypothesis would not work. By determining the range

of variability at any point in the growth rate curves for the F_2 generation, it was found that an asymmotote curve was obtained, thus indicating almost continuous variation in one direction only, the largest class being composed of those individuals with the smallest masses. The expected ratio of susceptible individuals in the hypothetical four classes was too far from expectation to be of any significance. Consequently, this conclusion was given up. It was then suggested that the F_1 hybrid individuals grew the transplant very rapidly by the presence of an "accelerator" introduced by the original non-susceptible parent. This conclusion was tested out by continually backcrossing susceptible individuals to the ancestral non-susceptible stock. It was found that there was a progressive slowing up of the growth rate as this process of backcrossing went on. By continued backcrossing to the original susceptible stock (the dilute browns) it was also found that there was a continued slowing up of the growth rate ensued. These data can be interpreted as follows: (1) The characteristic of transplantability is quite distinct from the characteristic of the proliferative energy of the neoplastic tissue. (2) The problem of growth rate is more complex and thus more difficult of analysis than the problem of susceptibility. (3) The tumor retains a constant reaction potential in these experiments both as to the phenomenon of transplantability and growth rate. (4) The variations of the growth rate in the various hybrid generations are probably produced by the genetic phenomenon of heterosis.

There is some indication that a large number of definite genetic factors may be involved in determined growth rate, since it has been possible to select out various substrains of mice that differ in their power of growing the same transplant at significantly different rates. I shall refer to the problem of growth rate later in this paper.

The next experiment consisted in the comparative study of two growths derived from the same mouse. It has been assumed that since neoplastic tissue has arisen by some process from normal tissue, it ought to have the same biological or genetic constitution as the normal

tissue from which it arose. Through the process of selection from heterogeneous mice, I was able to pick out several distinct genetic strains as far as the reaction to these two transplants was involved. One substrain was resistant to both transplants (— B — G); another substrain proved to be susceptible to B, but all the individuals were uniformly resistant to the other tumor (+ B — G); the individuals of the third substrain grew B progressively, whereas they showed a temporary tolerance to G (+ B re G); a fourth substrain contained individuals, all of which were susceptible to both transplants (+ B + G). Two points of genetic interest from this experiment will be pointed out. (1) If one assumes that one of these neoplastic tissues has the genetic constitution of the normal tissue from which it arose, then the other one can not have, since the two differ fundamentally in their physiological reaction to the same constant indicator. One is justified in concluding, therefore, that the genetic constitution of the tumor cell *may* deviate from the genetic constitution of the normal host tissue from which it arose, at least during the process of transplantation. (2) In one of the substrains, all the individuals spontaneously regressed one of the transplants, (G). Thus it is apparent that the failure of the transplant to grow in a particular individual is not the result of any peculiarity of the tumor tissue, but is the result of the biological constitution of the inoculated host. The host has the proper "soil" for the tumor to get a foothold but not sufficient for continued or progressive growth of the transplant. Spontaneous regression of the transplantable tumor as well as progressive growth is thus a genetic phenomenon and must be studied by genetical methods. May not the argument of dissimilarity between the spontaneous tumor and the transplantable tumor, based upon the presence of a high percentage of spontaneous regression in certain tumors in special cases, be artificial and not fundamental?

The last experiment I desire to present at this time has given two additional findings of biological interest and possible importance. The experiment consisted in the

continued transplantation of a certain carcinoma into a large number of F_2 individuals. I determined that for the successful growth of the transplant there must be the simultaneous presence of from five to seven Mendelian factors, possibly six. This constant reaction continued for about a year. There was then a change in the obtained results that could not be fully explained by chance alone. Several transplants were selected out and continued simultaneously. It was found that one of these subtransplants gave a two factor ratio; a second one gave a one factor ratio; a third one gave no indication of segregation of genetic factors, that is, it was non-specific; and fourth, the original tumor continued to give the same 5–7 factor ratio for some time. After carefully considering all the alternative hypotheses that may have a bearing upon this phenomenon, I was finally led to the conclusion that a genetic change in the nature of a mutation within the single tumor cell was responsible for the change observed. The term mutation is used in the broadest sense, "that is, any change or shift in the genetic or biological constitution of the tumor cell, which is perpetuated through the process of heredity (cell division)." Since these derived tissues had obtained different genetic constitutions through the process of mutation, during transplantation, we had obtained confirmatory evidence of a previous conclusion that the tumor cell may deviate from the genetic constitution of the normal tissue from which it had arisen at least during the process of transplantation. This phenomenon of the change in the reaction potential existing between the tumor tissue and the host has been confirmed in the case of six other transplantable tumors. One other point has come out of this last problem. The change in the reactivity of the cell has not only resulted in the changed transplantability character, but also has apparently affected the proliferative energy of the cell. There is a direct sequence of differences in the growth rate for the various subtransplants encountered in the experiment. Thus, the tumor giving a 5–7 ratio grew very slowly; the one giving a two factor ratio grew slightly faster; the one showing a one

factor ratio grew more rapidly; and the non-specific tumor in which no segregation of susceptibility factors could be demonstrated grew extremely rapidly. There is thus an indirect correlation existing between the degree of specificity of the tumor cell and the rate at which it will grow in a given individual.

From these experiments, we are able to arrive at some fundamental conceptions of the nature of the transplantable tumor tissue. In the first place, the behavior of the tumor tissue during the process of transplantation is apparently controlled by the internal or biological constitution of the tumor cell. Whether the fact that a large number of cells make up the tissue complicates the problem or not is of course not yet clear. But since the cell is the unit of structure of the neoplastic tissue as well as it is the unit of structure of normal tissue, it is more than probable that the physiological activity of the mass is controlled by the physiological activity of the single cell. The transplantable tumor retains an extremely definite reactive capacity for several years. This is apparently controlled by a well-established balance in the genetic make-up of the cells—this well-balanced condition of the cell being perpetuated by cell division from one cell generation to the next. A constant genetic constitution of the tumor cell results in a constant transplantability characteristic, as well as a constant proliferative energy component. Genetic changes in the internal constitution of the tumor cell occur sporadically during the process of transplantation. These genetic changes result in different physiological characteristics of the tumor mass—they change the transplantability of the tumor mass as well as changing the proliferative energy of the tissue. The genetic constitution of the tumor cell has deviated from the genetic constitution of the normal tissue from which it arose, presumably through the process of mutation, although the mechanism of change in this case has not been demonstrated. Thus, it is extremely probable that the problem of the neoplastic tissue is a problem of genetics and may be eventually solved by genetical methods.

CERTAIN ETIOLOGICAL FACTORS IN THE CAUSATION AND TRANSMISSION OF MALIGNANT TUMORS[1]

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THERE are few subjects in medical biology in so chaotic a state as is perhaps the greatest human problem of the moment—the cause and prevention of cancer. In most disease conditions, even where the cause or transmission is unknown, there are evidences which point unmistakably to an organism, a derangement or loss of function of some organ or gland of internal secretion, or of some toxic agent. These indications at least give the investigator some idea as to the direction in which he must launch his attack. The approach to the cancer problem is not indicated so clearly; and where some clues exist, they are often misleading and of a contradictory nature.

I will make no attempt to discuss malignant disease from the historical side with the various theories which have been promulgated from time to time, but will confine my remarks to the more recent findings developed from the experimental study of the disease.

What may be called the experimental era in cancer research started about twenty-five years ago with the discovery that malignant tumors of rats and mice could be transplanted from animal to animal. This discovery seemed to open the way for a direct investigation of the cause and the cure of the disease. Many important facts were brought out immediately. But the cause and cure of cancer was not among them. It was found that the cancer cell was limited in its transplantability, much as the normal cell is. The methods for production of so-called immunity to these transplanted cancers were soon found to be equally effective against transplants of nor-

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mal embryonic tissue. Perhaps the one distinction which could be drawn between the behavior of the transplants of normal and malignant tissue was that the latter had the quality of unlimited growth. Transplantation in both cases was confined to the species and frequently to closely related animals of the same species. There has been no direct evidence in this exhaustive study of mammalian tumors that the inoculated animal became infected with the disease in the sense that its own cells became transformed into malignant tissue. The inoculated animal acted only as a host, supplying blood vessels and supporting stroma, but finally becomes exhausted by the rapidly multiplying parasitic cells.

To sum up the facts gained from the earlier studies of the mammalian transplantable tumors, it was found that these could only be transferred from animal to animal by actual grafts of the living cells; that these cells retained their essential character even after years of transplantation; and finally that the inoculated animal supported the malignant growth, but no evidence has been found that the host's cells become malignant by contact with the inoculated tumor.

The next important advance was the data from the study of hereditary factors. It has been found possible by selected breeding to develop families of mice which spontaneously develop cancer in a very high ratio. Whether these hereditary factors follow the Mendelian law or not is a disputed point, but it may be said that heredity plays an important part in the development of spontaneous cancers of mice, for it is possible to select families which rarely if ever develop the disease, or families which develop it in a very high rate. It may be said in passing that there have been no evidences of contact infection with the disease. Mice from a non-cancerous family, nursed by a cancerous mother, do not become subjects of the disease. A group of families giving different cancer rates kept in the same breeding room under the same conditions have not shown any tendency for the non-cancerous stock to become cancerous.

The next point brought out by the experimental school was the relationship of chronic irritation to cancer. The feeling has long existed that if it were possible to produce cancer experimentally we would be well on the way to the discovery of the cause. The experimental production of cancer was finally achieved several years ago by Febiger and shortly afterward by two Japanese investigators, Yamagiwa and Itchikawa. With the perfection of the method utilized by the last investigators, it is now possible to produce carcinomas and sarcomas more or less at will. This result has led some investigators to consider chronic irritation as the sole cause of cancer, and it has been suggested that repeated scarification of the skin or repeated pricks with a needle would result in cancer, but so far this contention has not been sustained. The irritants causing cancer are confined to certain coal tar derivatives, X-ray and radium, and some simpler chemical substances, and to irritation of certain parasites. The relationship of X-ray and radium burns, chronic coal tar irritation, soot and certain parasites to malignant disease in man has been known for many years. That the result here is not due to a simple chronic irritation is indicated by the fact that at least three of the agents mentioned cause besides a local irritation a general lowering of resistance to the transplanted cancer. This would seem to indicate that the local reaction must be accompanied by a general effect on some mechanism of control before cancer develops. Again, this method had failed to give us the final solution of the problem.

Finally, I would like to review briefly the information gained from the study of sarcomas of the chicken which at the present represents a unique group among malignant animal tumors. A study of these was first undertaken at the Rockefeller Institute. These tumors seemed to fulfill every criterion necessary to classify them as true malignant tumors. They suffer the same limitations in transplantability as do the tumors of other animals in that they will grow only in the same species in which they

originally occur. Among these tumors transplanted and studied were two spindle-cell sarcomas, a myxomatous type of sarcoma, and a tumor which was designated an osteochondrosarcoma. The startling finding in regard to these chicken tumors was the fact that they could be transmitted from chicken to chicken by a cell-free filtrate, or the dried powder of the tumor. The tumor propagated in this fashion retained all the cell specificity and peculiarities of the parent tumor. Here we had three cell-free extracts, one injected into a chicken caused the connective tissue to become malignant with a characteristic spindle-cell type, another extract induced the connective tissue to develop into a myxomatous type of sarcoma, and a third extract caused this tissue to differentiate into bone, cartilage and bone marrow. These findings have been variously interpreted as indicating the presence of a so-called filtrable or ultramicroscopic living organism, or indicating an agent more closely related to the cell, something in the nature of an abnormal growth enzyme. Exhaustive study of these tumors over several years failed to establish either of these points of view.

Recently an additional study of the chicken tumor has appeared, but this work is too fragmentary and too recent to warrant any final judgment at the present time. It has been received by cancer investigators with reservation. I refer, of course, to the work of Doctors Gye and Barnard of London.

These investigators find that if a piece of chicken tumor is placed in culture media and incubated in the absence of oxygen, the power of the extract to produce tumor is lost after a few days. If, however, the clear, supernatant fluid from these cultures is mixed with fresh extract of tumor in which the tumor producing agent has been killed by chloroform, this mixture is capable of producing a typical malignant growth in chickens. They assume from this finding that two factors are necessary for the production of malignant tumors: first, a microorganism which they can cultivate in the test-tube and which they claim to

have isolated from rat, mouse and human tumors, as well as from the chicken tumor; and second, a specific chemical factor which determines the species and the tissue which will be attacked by the living organism. So far this specific chemical agent has been derived only from the chicken tumor.

I do not wish to indulge in hypothetical destructive criticism of Gye's work, for he has undoubtedly made an important contribution; but it must be said that as the work stands at the present time it is open to entirely different interpretations than the one he places on it. In addition there is an unfortunate lack of controls, which makes one rather hesitate to accept the observations without reserve. For example, in his subcultures of the chicken tumor he always adds a generous supply of fresh embryonic tissue. It has long been known that embryonic tissue may assume a malignant character under the influence of the filtrate from the chicken tumor and in turn these cells are capable of producing more of the active agent. This point has not been considered by Gye. Nor has he considered the possibility of actual survival of the tumor cells in culture. Here he takes for granted that the tumor cell will not survive in the absence of oxygen, but Warburg has shown that tumor cells may carry on active metabolism in the absence of oxygen if they are provided with sugar. From our own personal experience, we know that tumor cells may continue to actively multiply in the culture media used by Gye for at least five days in the absence of oxygen. These are only two examples among several omissions which might be mentioned and which may or may not prove of importance in the final analysis.

Let us ignore for a moment the deficiencies of the experiments and consider the conclusions which Gye has drawn, based on the fact that the agent from the chicken tumor after being carried through a number of subcultures is still able to cause a typical tumor of the chicken after being mixed with a specific chemical factor. He con-

cludes that this is proof of the presence of a living organism. A few years ago this deduction would have been considered justified, but now we know of the existence of certain agents more closely allied to the enzyme group which give just as good evidence of multiplication as does this agent of the chicken tumor. You are all no doubt familiar with the bacteriophage which gives every evidence of increasing when brought in contact with bacteria —yet there is very strong reason for believing that this is not a living, formed agent. The enzyme which causes sweet oil to become rancid seems to have no limit in the amount of its reaction. Pancreatic extract activated by enterokinase may be carried for thirty generations of what would correspond to subcultures and still be found as active as in the beginning. Many other examples of a similar nature could be mentioned.

It might be considered that Barnard's work proves the presence of a formed organism in connection with malignant tumors, but so little is known about these so-called ultramicroscopic bodies that one would be quite unwilling to accept the evidence as it stands to-day. With the magnification used it is possible that even some of the larger conglomerations of protein molecules would give the appearance of formed bodies. In any case, observations of this nature will require perhaps years of careful analysis with parallel biological experiments before any final judgment can be passed on them.

Can any theory of the cause of cancer be formulated at the present time? Even assuming that we are prevented from transmitting the mammalian tumors with a filtrate by some slight technical difficulty, and that transmission of cell-free filtrates is a characteristic of malignant disease, could we assume a living organism as the cause? It would be necessary to assume that the organisms are ubiquitous, for we know that with coal tar we can induce malignant tumors in practically 100 per cent. of mice and probably equally frequently in other animals. How could we explain an organism so varied in its effect as to

cause the connective tissue to differentiate in three different directions, as we have seen the filtrate from the chicken tumors perform this remarkable feat? How could we explain the high degree of tissue specificity of the tumors on transplantation? What could we say about the inherited tendency? Is this to be assumed as an inherited susceptibility to an infection which is always present? Even admitting a specific chemical factor as a necessary accompaniment to the infecting organism, it would strain the imagination to fit this conception to the facts as we know them.

The theory of the absence of oxygen as causing the development of an abnormal metabolism of the cell brought forward by Burrows as a result of his tissue culture studies and also suggested by Warburg from his metabolism studies is still without sufficient proof. Till normal cells are definitely transformed into malignant cells by depriving them of oxygen, this theory can not be considered seriously. The numerous other theories which have been brought forward from time to time need not be discussed here on the grounds of inadequate supporting proof. We are forced to the conclusion that no theory can be formulated at the present time. But may I suggest as my belief, that when the cause of cancer is discovered it will be found to be some agent or force most intimately associated with the mechanism of the growth of the cell and will be a discovery of equal importance to those studying normal cell phenomena and to the cancer investigator.

THE RÔLE OF FUNCTIONAL ACTIVITY IN THE PRODUCTION OF MAMMARY CARCINOMA[1]

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THE preceding papers of this symposium have covered very adequately the historical field of experimental cancer and the theories of tissue activation and continued growth. I shall confine my remarks to a narrower field, one that concerns certain functional disturbances that apparently underlie the production of cancer in the mammary gland of the female.

In the history of progress in medical research the sequence of events is usually from the experimental laboratory to the clinic, but I must confess that the main promptings and ideas behind the experiments I am about to describe came from my association with the clinical study and treatment of human cancer. I am particularly indebted to Dr. James Ewing for calling attention to the fact that in a considerable number of women suffering from carcinoma of the breast there was evidence of a previous functional disturbance in the mammary gland. The obvious question to be determined was whether or not the disturbed function itself bore a causative relation to the production of the disease.

With human patients it was possible to do little more than collect statistical data and to group the results according to clinical histories. It occurred to me that it would be worth while to subject a series of female laboratory animals to various methods of breeding and to limit their opportunities for suckling their young.

About two years ago I selected certain female albino mice from a strain that I had had in my own laboratory for eleven years. Their homogeneity had been purposely

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increased by careful inbreeding, which consisted in mating only the most healthy and active individuals in each generation. It was found that mammary cancer appeared in only about 5 per cent. of the females of this line who were breeding normally and suckling after each pregnancy an average-sized litter of young. The tumors that did appear became evident only in females from eighteen to twenty-four months of age. The animals were large, vigorous prolific individuals and suckled their young successfully.

Females from the above strain were divided into two groups: one serving as a control was allowed to breed normally, *i.e.*, the females were remated only after nursing their litters for about six weeks. The test animals from the same family were then bred under the following procedures designed to subject the mammary tissues to abnormal conditions:

(1) Females were bred when very young (two to three months old), and their litters removed immediately after each parturition, or not more than twelve hours later, and the mothers remated at the oestrus that promptly follows parturition. The mammary glands in this group were denied what might be called the maturing influence of at least one period of normal suckling.

(2) Females were bred for the first time when from six to nine months of age, and treated as in the above group. In these animals the factor of age was added to the conditions described previously.

(3) Various combinations were made of alternating periods of suckling of one litter and non-suckling of the next. The mammary glands of these females were allowed to become fully developed before dysfunction produced the desired stagnation of retained secretions.

The time at my disposal does not permit of more than a general outline of the results so far obtained. I shall mention as an example the incidence of mammary carcinoma in a group of fifteen test animals, as compared with the average of a large number of control individuals.

Animals of Group 1 have bred very rapidly, having had as many as nine to eleven consecutive litters, with an average of only twenty-four days between the dates of parturition. In this group mammary tumors have appeared at an early age, *i.e.*, when the females were from eight to eleven months of age.

The occurrence of fully developed mammary cancer in such young animals is one of the most interesting features of these experiments.

The following breeding record is a typical example of an animal breeding with remarkable regularity and prevented from suckling its young.

FEMALE A—BORN FEBRUARY 6, 1924

Litter No.	No. of young per litter	Date of birth	Nursing history
1	9	Apr. 29, 1924	No suckling
2	7	May 30, 1924	" "
3	11	June 30, 1924	" "
4	10	Aug. 2, 1924	" "
5	9	Aug. 22, 1924	" "
6	10	Sept. 11, 1924	" "
7	8	Oct. 1, 1924	" "
8	8	Oct. 21, 1924	" "
9	7	Nov. 10, 1924	" "

Female A was only two and three fourths months old when her first litter was born. During the following seven months it gave birth to nine litters, totaling seventy-nine young. This record was made possible because of the great care given to the diet and general housing needs of the breeding females.

Coincidentally with the last parturition a tumor was noted in the inguinal region of the mammary gland on the left side of the body. On histological study this was found to be vascular, alveolar, cellular carcinoma, with multiple foci of origin.

About a month later, when the female was again pregnant, a second independent tumor was noted in the anterior mammary region on the left side of the body.

A tenth litter of three young was born on December 18, 1924, at which time there was a local recurrence of tumor growth in the inguinal region previously operated upon. The tumor when removed showed more malignant carcinomatous changes and the cervical tumor was also found to be carcinoma on histological study. An eleventh litter of two stillborn young was born on January 10, 1925, despite the trauma of the two previous operations. Two days later a small tumor was noted in the thoracic mammary region on the right side of the body.

Here, then, is an animal that shows that a considerable portion of the mammary gland was what one might call condemned to tumor growth by the abnormal functional activity impressed upon it by experimental means.

The females in Group 2 were not allowed to breed until they were from six to nine months of age. They then were bred, but were prevented from suckling their young. It was found that the age of tumor incidence in this group was from eleven to twelve months. In this group a relatively low average of only four periods of pregnancy, followed by the prevention of suckling, precedes the onset of the tumors.

Animals in Group 3 show on the whole that, when periods of suckling alternate with periods of non-suckling, the tumors appear after fewer consecutive litters, as compared with the condition in group one.

Prevention of proper drainage of the mammary gland has resulted in marked stasis of the mammary ducts. This is associated with the retention of a considerable amount of decomposing, stagnated and probably chemically altered milk, which it is possible was sufficiently irritating to cause considerable disturbance within the gland.

Contributory evidence was given to this view by the histological study of serial sections of a considerable portion of the mammary gland in mice with very early but definitely established mammary carcinoma. The cyclic relation between ovarian and mammary systems was ob-

viously deranged by the experimental methods employed, and the glands in rapidly breeding, non-suckling females were not able to properly reach a normal resting condition for any considerable period of time.

In mice breeding rapidly from an early age (Group 1) and not allowed to suckle their young, the mammary glands were apparently prevented from reaching their normal degree of development; and it took a considerable number of consecutive litters, followed by periods of non-suckling, before the onset of tumor growth. Whereas, in animals older when first bred (Group 2) or in Group 3, where the suckling at one period definitely established large, well-developed glands to start with, it took fewer periods of pregnancy followed by non-suckling before tumors became evident.

The tumors have appeared coincidentally with, or a day or two after, parturition. The animals have grown tumors and embryos at the same time.

More recently an attempt has been made in a separate group of animals to produce, by a minimum of traumatic disturbance, an artificial stasis in the mammary gland. This was attempted as a check upon the results described above. The ducts on one side of the body were operated upon and ligatured close to the nipples. The animal was then bred and allowed to suckle its young on the opposite side of the body.

The preliminary results in this part of the work have been gratifying. In six of the animals so far observed three are still living, twelve independent mammary tumors have been noted and have all occurred on the side of the animal which had been previously operated upon. One female has had as many as four independent tumors. These have occurred adjacent to four out of the five nipples near which the mammary ducts have been ligated.

Histological examination showed the presence of solid mammary carcinoma, associated with marked stasis of the duct system on the ligated side of the body.

The tumors that appeared in the four groups of experimental animals were definitely malignant in nature, with

a rapid rate of growth and a marked tendency to local recurrence after operative removal. The breasts have usually shown evidence of multiple foci of tumor growth.

In the control group of animals containing females breeding regularly and suckling their young, the incidence of tumor growth is less than 5 per cent., and the tumors make their appearance only in animals between about eighteen to twenty-four months of age. In the first group of fifteen animals, in which a dysfunction of the breast was brought about by non-traumatic means, thirteen females or 87 per cent. developed mammary carcinoma at comparatively early stages as described above.

I find that we are particularly indebted to Leo Loeb for calling attention to the apparent association between ovarian and mammary gland activity in relation to carcinoma in the latter organ. His experiments throw light on the subject from a different angle. It was found that castration of female mice below six months of age leads to a marked decrease, 60 per cent. to 9 per cent., in the incident of mammary carcinoma, while similar treatment of females above six months of age was without effect. Similarly, but to a less degree, prevention of breeding in female mice decreased the incidence of mammary carcinoma and increased the cancer age. Loeb found that in the non-nursing rat at the second day after labor the ducts of the mammary gland were dilated by the stagnating secretion.

In conclusion, I would like to call attention to the fact that the results of my own experiments are of interest in comparison with those of other investigators who have attempted to produce true mammary carcinoma by various traumatic means, insertion of foreign bodies into the glands, etc., and despite a very ingenious assortment of insults, their results were uniformly negative. With these results in mind, the relatively simple procedures I employed appear more significant.

RECENT CANCER RESEARCH[1]

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PRESUMABLY I have been asked to speak to you because I was in England and Germany last spring and met Gye and Blumenthal, sent the first account of Blumenthal's work to *Science* and brought back the first news of Gye's work, which was given to the cancer workers of the United States at their annual meeting, May 4, and printed in *Science* June 12, a month before the newspapers heard of it. It is the only big scoop on newspapers I ever expect to make. Gye's first paper was published in *The Lancet* July 18, 1925. I had long conversations with these two gentlemen, was in their laboratories, met their friends and scientific coworkers and was invited to their homes.

My interest in cancer dates from the time when in crown-gall, a tumor of plants due to bacteria, I found, or thought I found, many resemblances to human and animal tumors. That was twenty years ago. From the various analogies I reasoned that animal and human tumors must also be due to parasites.

Twenty years ago Ribbert's ideas were in vogue and nobody believed any longer that cancer could be due to parasites. The idea was laughed at or received with incredulous smiles. To understand Ribbert's ideas we must go a little farther back. In the second half of the nineteenth century Rudolph Virchow, a great German pathological anatomist, sanitarian and publicist, propounded the idea that cancers must be due to prolonged irritation of various sorts. This view was received with great favor at first but was gradually supplanted by Cohnheim's theory, according to which cancers are due

[1] Address given at the symposium on the Cancer Problem, American Society of Zoologists, December 30, 1925.

to the growth of tiny fragments of tissue displaced during embryonic life, that is, cut off from the mother tissue and embedded in other tissues where they remain dormant until they develop as a tumor. Favoring this hypothesis is the well-known fact that owing to accidents prior to birth tiny fragments of organs are often found embedded in other tissues. Opposed to it are many observations, accumulated gradually, such, for example, as the fact that with tar we can produce a tumor in any part of the body. Neither Cohnheim nor any of his followers were ever able to explain just why tissue fragments, twenty, thirty, forty, fifty, sixty, seventy, eighty, even ninety, years dormant, should suddenly begin to grow uncontrollably. Furthermore, all subsequent observations have shown that embryonic skin, hair, liver, bone, muscle, etc., separated from the parent mass tend to grow out into mature cells, unable to proliferate.

This brings us around again to Ribbert and his modification of Cohnheim's theory. He conceived the idea that mechanical displacement of tissues leading to tumor-formation might take place at any time of life. Always for him, however, connective tissue first multiplied cutting off some fragments of epithelium, which, separated from its fellow cells, begins to grow abnormally and uncontrollably. Ribbert held a responsible university position and was a very distinguished man. By skilful use of the spoken and written word, fully illustrated by drawings, he convinced pretty nearly everybody in the early part of this century that he was right. It needed, he said, no parasite to explain cancer, only a dislocation of tissues, followed by connective tissue proliferation. But his theory did not make it clear why there are innumerable wounds always with more or less dislocation of tissues in which cancer never develops. More and more during the last twenty years, as all sorts of epithelial cancers (carcinomas) have been studied under the microscope, investigators have come to believe that epithelial tissues and not connective tissues are the first to become

abnormal and cancerous, and that the stimulus to connective tissue is only secondary and sometimes altogether wanting. Ribbert's ideas, therefore, are not any longer in favor. They have gone the way of Cohnheim's original theory.

Meanwhile the recent phenomenal success of many workers in the production of cancers by means of tar, soot, arsenic, etc., have served to revive Virchow's irritation theory, and this theory is now in great favor with many persons, with all persons, it may be said, who have discarded the parasitic theory.

It is worth while, therefore, to explain just what has been done with tar. Chimney sweep's cancer has been recognized for more than one hundred years. Cancers have also been observed to be unduly prevalent among workers in arsenic, anilin and paraffin. These facts, which there is no gainsaying, led many persons to attempt to produce cancers in experimental animals by means of soot, tar and other substances, but all the early experiments failed. Not until two Japanese students of cancer, Drs. Yamagiwa and Itchikawa, patiently tar-painted rabbits' ears every other day for a year were distinct cancers produced. Their first experiments were begun in 1913. Since then these experiments have been repeated many times in many countries on various experimental animals with positive results. Rats, rabbits and guinea pigs proved rather resistant; mice on the other hand were found to be very susceptible to the tar painting, especially certain strains of mice, a matter to which I will refer again later.

With susceptible strains of mice and a good tar or a bad one, if you prefer, cancers in great numbers have been produced within a few months' time, the skin being tarred in the same spot every second or third day. Many of the animals, of course, die of intercurrent diseases, but of those that survive 50 per cent. or more develop cancers. Once started, the cancer continues to grow even though the tarring is stopped. The progress of the dis-

ease is about as follows: The skin thickens, the cells enlarge, the hair falls off, papilomatous warts develop and finally open sores, some parts of which, at least, end in becoming invading tumors. Not all tars are active, nor are all mice susceptible.

Similar results have been reported from the use of extracts of soot prepared in various ways, and from various other chemicals. But, first of all, one must have sensitive animals. Bonny, in Amsterdam, told me that the same tar they had used very successfully on Dutch mice failed to produce tumors on mice at a distance in the laboratories of other workers. He also told me that he commonly finds protozoans in the ulcers of his tar-painted mice, which he has not observed in the normal mice. I am inclined to think, therefore, for this and other reasons, that tar-paintings and other irritants are not the actual causes of cancer, but that the open sores they produce are the gateways through which some undetected common parasite enters. Anything is liable to get into a wound kept from healing for months by the repeated application of irritants. All the tarred skin does not become cancerous, but only tiny spots here and there. This has to be explained by those who think that the cancer is due to the direct action of the tar. Moreover, there are skin parasites on most of the tarred animals and often the rooms in which they are kept are very dirty and swarming with flies, which I have seen visit the wounds.

The two commonest kinds of cancers are carcinomas, which are abnormal growths of the epithelium of the skin, mucous membrane and glandular tissues, and sarcomas, which are growths of connective tissues. Formerly these two types were supposed to be quite independent in their origin and progress and generally they seem to be, but sometimes both tissues are stimulated and the result is a mixed tumor, a carcino-sarcoma. Moreover, in tumor grafting, which has been done very largely during the last twenty years on experimental animals, especially on mice, a pure carcinoma has been

known to give place in the course of successive graftings to a mixed tumor, or to a pure sarcoma. Also in the tar-paintings mixed tumors are not infrequent. By some these have been called carcinomas, masquerading as sarcomas. I should also mention here that "benign" tumors are those that do not form daughter tumors, and "malignant" tumors are those that do form distant colonies, or metastasize, as we say technically. In the latter the primary tumor may be of small consequence compared to the secondary tumors which are often located in vital organs and grow rapidly with disastrous consequences.

The parasitic theory revived and took a great stride forward when Dr. Johannes Fibiger, the pathological anatomist in Copenhagen, produced numerous cancers (carcinomas) in the fore-stomach of rats by feeding them a nematode taken from the muscles of a cockroach. His first paper was in 1913. This cockroach lives in the West Indies and is found only exceptionally in Europe around sugar factories, or on ships engaged in the West Indian trade. His results, now accepted by everybody, have been explained on the irritation theory by most workers, but not all the rats develop the tumors. In some of them the feedings result only in a violent inflammation of the throat and stomach, which eventually subsides. I explain these results by supposing some of the nematodes to be carriers of an invisible tumor-parasite and others not. More work needs to be done. Fibiger was the first man to produce a carcinoma experimentally by means of a parasite. I have seen Fibiger and talked with him and have the utmost confidence in his researches, which covered a period of more than half a dozen years.

Following close upon the heels of Fibiger's brilliant work came the equally brilliant work done by Bullock and Curtis under the direction of Dr. Francis Carter Wood at the Crocker Laboratory for Cancer Research, connected with Columbia University in New York City. Here they have produced more than a thousand malignant tumors (sarcomas) in the livers of rats by feeding

them the eggs of a tapeworm of the cat. The eggs hatch and the tiny worms find their way into the liver of the rat, often in considerable numbers. Around each worm the connective tissue cells of the liver produce a fibrous wall, making a cyst in which the worm lives and grows. This cyst is often as large as a small cherry. I have seen rat livers full of them. Each cyst contains a living worm. When such rats are eaten by cats, and especially by kittens, the larval worms change in the cat's intestine to the perfect egg-laying Taenia. In the wall of the rat-liver cyst the malignant tumor begins. It does not start in all parts of the cyst wall at once, but rather it begins in one or more tiny areas. Once started, it grows rapidly and soon destroys the animal, metastasizing freely to other parts of the body. Here again there may be several to many cysts in the rat's liver, each occupied by a larval worm but only very rarely do more than one or two of them become sarcomatous, generally only one. This has to be explained. Moreover, not all the rats that have been fed the tapeworm eggs develop tumors, nevertheless by breeding together sarcoma-bearing animals it has been possible to obtain rats so sensitive that 100 per cent. are susceptible to the tumor, but only when they have been fed with the eggs of the tapeworm. They have the tendency, but a second factor is necessary.

The varying susceptibility of different rats and mice brings us face to face with the question of heredity. More and more as the subject is inquired into heredity is seen to play a distinct part in susceptibility to cancer. A dozen or more different observers have reported very interesting observational and experimental data. Miss Maude Slye, of Chicago, has, perhaps, done more animal breeding with this end in view than any one else. She has obtained strains of mice entirely resistant to cancer; and without any tar-paintings or inoculations of any sort, other strains in which 100 per cent. become cancerous as they grow old. Marsh, in Buffalo, has obtained similar results with mice, and I could mention other persons.

Various physicians have denied strenuously that this applies to man, but I believe it does. The laws of heredity are universal from the lowest form of life to the highest. Moreover, Professor Warthin, pathological anatomist of the University of Michigan, has traced the incidence of cancer in a German family settled in Michigan and has shown an enormous excess of cancer in the descendants of a man who died of cancer of the stomach. His records extend through four generations. In this man's descendants the males usually develop cancer of the stomach and the women, cancer of the uterus, and earlier each generation. Of the eighty-eight adult members in this line, *i.e.*, those who have reached cancerous age, twenty-eight (31.81 per cent.), have developed malignant tumors. You will find his last paper in the June, 1925, number of *The Journal of Cancer Research.*

I can not think that heredity is the only factor in cancer, but I am convinced that it is one factor. There may be several other factors. One other factor must be, I think, either a long-continued irritation, due to a great variety of causes (the most generally accepted theory at present) or else the specific action of one or more parasites.

Before I touch on the question of parasitism I wish to say a few words on the cancer cell. It is, of course, as you all know, a body cell, which the body can no longer control, and which multiplies inordinately at the expense of the rest of the body. The prime question here is what makes the tumor do this. Is the stimulus to this malignant overgrowth intrinsic or extrinsic? Does it come from within the cell, due to some change in its internal mechanism brought about by a parasite or by some non-living irritant, or is it due to a changed environment acting on an otherwise normal cell? Here is the problem that lies just ahead of us. The cancer cell has been said to be only a normal cell growing rapidly because inhibitions have been removed, but in opposition to this view it is known to be slightly more sensitive to outside influences, X-rays,

radium emanations, lead salts, etc., than normal cells. Fischer, of Copenhagen, has also shown that a single chicken sarcoma cell will grow in serum cultures and form a colony, whereas a single normal connective tissue cell under the same conditions will die. Alexis Carrel, of the Rockefeller Institute (*Jour. Am. Med. Assoc.*, January 17, 1925, pp. 157–158), has shown by means of tissue cultures that the macrophages (the large mononuclear leucocytes), supposed to be scavengers and destroyers of infection, pick up and become carriers of the virus of the chicken sarcoma. Warburg, in Berlin, has shown both for carcinoma and sarcoma that the malignant cell breathes anaerobically, even in the presence of air, whereby sugar is split and irritant lactic acid is produced in the tissues continuously. In all these ways the tumor cell behaves unlike a normal cell, but we can not decide from this whether the stimulus to growth is due to a parasite; to some derangement of the internal mechanism of the cell due to an irritant; or, finally, to some change in the environment of the cell. We might suppose some of the chromosome destroyed, so that all descendants of the given cell or cell complex would necessarily be abnormal. Warburg has another very ingenious and seductive theory, which I will touch upon later, and Burrows has a third somewhat similar explanation.

I come now to very recent studies dealing with the question of parasitism. I will first take up Blumenthal's work, which was stimulated by my work upon crowngall. Dr. Blumenthal is the responsible editor of the German cancer journal, *Zeitschrift für Krebsforschung,* the oldest and most influential cancer journal in the world, and is director of the Cancer Laboratory of the great Charity Hospital in Berlin (Universitäts Institut für Krebsforschung). Previous to his study of cancer he was interested in changes of kidney secretion due to a great variety of diseases and wrote a book on that subject. I threw down the challenge so peremptorily years ago that the Germans finally began to study the crowngall, but

with no idea that plant cancer was in any way related to animal cancer, except *in name,* as one of them said. I sent cultures to Germany and after several of them had worked five or six years on the subject, Dr. Blumenthal became convinced that I might be right. What led to his complete conversion was the finding of an organism in cultures made from a human breast cancer, the colonies of which looked like those of *Bacterium tumefaciens,* the crowngall parasite. This was an isolation made by Paula Meyer, one of the laboratory assistants. She had been working with crowngall several years and knew the appearance and behavior of the organism very well. She had never before seen anything like it in cultures made from human cancers and the resemblance was so great that she subcultured the colony and inoculated it into sunflowers and tomatoes, obtaining numerous tumors on these plants. Rats were then inoculated with it, first alone, and then mixed with sterile diatomaceous earth. In both cases they obtained small tumors, but these tumors receded and could not be transplanted into other animals. Subsequently they obtained transplantable, metastasizing, malignant tumors, by adding heated cancer serum to the bacterial culture and the kieselgur when making inoculations, and one of these tumors has now been carried through more than a dozen generations of transplants by inoculating minced fragments into other animals. I have also seen a long series of transplants of this tumor made in the Serum Laboratory in Dresden.

The first tumor is known as *PM* or gamma. In some of the metastases it looks like carcinoma, in others like a sarcoma. I think it may have originated from endothelium, but its tissue origin remains uncertain.

The Berlin Cancer Laboratory of Dr. Blumenthal has adjoining it a small special hospital for incurables from which to draw material. There were about a dozen cancerous patients in it at the time I saw it, and there is a frequent change, old patients dying and new ones taking their place. Considering their torment, it is the nearest

to hell of any place I have ever been in. They have cultivated suspicious bacteria from at least a dozen of these patients, and with a half dozen or more of them they have produced tumors in rats, but owing to the small size of their animal house and to their limited income, allowing only a few assistants, and requiring all sorts of expenditures to be made on a very small scale, they have been able to test out thoroughly only a few of these tumors. Why does not some one rolling in luxury give them money? They need it badly. Money could hardly be put to a better use, and so much is wasted in selfish indulgence all over the world! At the present time they have three good transplantable, metastasizing, malignant rat tumors—*PM, L* and *Delta*—obtained with bacterial cultures taken from as many malignant human tumors, the first two from inoperable breast carcinomas. With cultures of *PM* two transplantable and metastasizing rat tumors of the same type have also been obtained in Dresden at the Serum Laboratory and carried through a long series of transplants. *PM* is the schizomycete that looks most like the crowngall organism on culture media and reacts serologically like it. I mean by this latter statement that the serum of rabbits which have been dosed with *PM* flocculates *Bact. tumefaciens* (hop strain) and *vice versa,* the serum of rabbits inoculated with *Bact. tumefaciens* flocculates cultures of *PM.* This strain, *PM,* taken (be it remembered) from an inoperable human breast cancer, grown and subcultured on agar, gave as good crowngalls on sunflowers when first isolated as I have ever seen or obtained with *Bact. tumefaciens,* and after two years on culture media it was still able to produce tumors in sunflowers, as I saw when I was in Berlin. I did not see any sunflower tumors produced with *L,* although young plants were inoculated with it while I was there, so that if it ever had tumor-producing power on plants it must have lost it, a not uncommon occurrence with our isolations of *Bact. tumefaciens.* Indeed, we have up to this time found only one strain of the crown-

gall organism which has preserved its virulence unchanged through a long period of years, namely, the hop strain, isolated twenty years ago. All the other isolations have lost virulence sooner or later, and usually within three or four years. The rat tumor called *Delta* has been obtained since I left Berlin and I know of it only through correspondence. I understand it to be a transplantable, metastasizing tumor obtained with a bacterial culture made from an inoperable uterine cancer in a woman. None of these bacteria are exactly like any isolation of crowngall we have ever made in this country, nor are they identical with each other. They exhibit a considerable number of cultural and other differences, but they all cause rat tumors (I am speaking of the three sorts, *PM, L* and *Delta*) unless we are to assume with Reichert that the tumors are not due to what we see but to some invisible virus carried along with the bacteria as a contamination or as a symbiont, something suggested by Gye's work, and suggested by me much earlier as a possible explanation for loss of virulence on the part of a variety of plant parasites ("Bacterial Diseases of Plants," 1920, p. 477). This, of course, is possible, and Dr. Blumenthal has left it an open question, especially in his later papers, although in his first long paper in the *Zeitschrift,* he posited *Bact. tumefaciens* as the type of a whole series of tumor-producing bacteria, some of which cause tumors in man and the lower animals. Another curious thing is that a second factor seems to be necessary to cause his rat tumors, that is, a sensitizing chemical substance, namely, heated cancer serum. This, in a way, connects his studies to those of Gye in London, who also finds a chemical factor necessary. Blumenthal's rat tumors are unlike any others I have ever seen. They often grow to large size, metastasize freely, chiefly in the glands of the groin and axilla, but I have seen metastases in the liver, around the heart and in the lungs. Sometimes the lungs (in *PM*) are full of metastatic nodules. This is the first time that any ani-

mal tumor has been produced with bacteria cultivated from a human tumor, and carcinoma at that!

Also in the studies made at the Serum Laboratory in Dresden, while they obtained, as they told me, the two rat tumors with cultures of *PM,* without the use of cancer serum, they did actually use a second factor, judging from Dr. Reichert's printed paper (*Deutsche Medizinische Wochenschrift,* August 7, 1925), because in addition to the bacteria and the kieselgur he put into the other side of the rats some of the minced *PM* tumor. We must wait for repetitions of Blumenthal's work in other laboratories, the world over, before we can know definitely the full extent and value of his contribution and the same is true of Gye's work. In some ways the Berlin work appears to be contradictory of Gye's work in London, but perhaps it is not really so. One of the questions at issue is whether the rat tumors are really due to the visible bacteria cultivated from the human cancers, or to something else introduced along with them. Another is whether tumors of mammals may not be due to quite other causes than those of birds.

Gye, in London, working on Rous's chicken sarcoma, was the first person to cultivate its virus. That he did through a series of tubes of KCl broth plus rabbit serum and from the last tube he was able to produce the sarcoma in 50 per cent. of his chickens. This he was able to do, not with the virus alone, but only when to his clear fluid cultures he added a second factor, namely, chicken-tumor juice in which he had destroyed the virus by means of chloroform. Neither fluid would produce the tumor by itself, but numerous tumors were produced from mixtures of the two. At the time I was there he had obtained forty tumors in this way. He was also able to show that the virus is particulate by means of the centrifuge, the centrifuged precipitate being infectious and the supernatant liquid non-infectious, or only slightly so, although nothing was visible in any of the cultures stained or unstained, either to the naked eye or under

the highest powers of the microscope. He regards the virus as non-specific and the chemical factor as narrowly specific. He was much puzzled in April, so he told me, because while he could get 100 per cent. of infections by transplants of the fresh tumor to healthy chickens, he could get tumors with cultures of his invisible virus in only about one half of his chickens. I said to him: "You undoubtedly have *attenuation of virus and a vaccine,* just as Pasteur had in case of his chicken cholera, and those chickens which have not developed the disease should now resist the strongest virus." He did not tell me everything, but I could see quite clearly that he was thinking very seriously along the line of the possibility of a vaccine and making many experiments, in fact he was working night and day on the problem, testing out his cultures at all temperatures from 35° to 42° C. with attenuation in mind, as he admitted to me the following day. He told me at the time about obtaining the chicken tumor with virus from a mammalian sarcoma, but asked me not to say anything about that part of his work. I see that the newspapers have announced recently that he has said that he has obtained a vaccine. If he has really said this, then he has got it.

Dr. Gye is a well-balanced, industrious and quiet man, thirty-five or forty years old, with an excellent training for the work he is doing. He impressed me as a keen critic, cautious and well aware of the pitfalls on every hand, and more anxious to be right than to be famous. Like most oncologists, he formerly believed that all malignant tumors were non-parasitic, but now he believes that all or most of them are due to parasites. Since the great newspaper furore over his discoveries, he has written me that if he had realized what a commotion his announcement would make he would have withheld it another year. I urged him to publish promptly. He has recently written me that his work is going on steadily and, so far, satisfactorily. Dr. Barnard's part of the work has been to invent a microscope with new optical

properties by means of which one can see and photograph the organism. A lot of nonsense has been written about him in our newspapers. What does it matter whether he is the son of a hatter or of a king!

His microscopic observations have been made with light taken from the middle of the spectrum. There is, he told me, a trifle of orange in it, but it is a nearly monochromatic green band light taken from a mercury lamp. The organism is large enough to be seen with ordinary microscopes using high powers and the reason it is invisible is because it has the same refractive index as the serum in which it is grown and the optical parts of our ordinary microscopes. Even with Dr. Barnard's microscope the *contents* of each little globe is invisible. Only the periphery stands out clearly.

Now that money has been appropriated to give Gye and Barnard suitable buildings and much needed assistants, we may expect many interesting results from the Mill Hill, Rhodes Farm Laboratory. Dr. Gye, I understand, has been twelve years out of the medical school; during the war he was on the French and Italian battlefront four years, occupied in the suppression of infectious diseases, and toward the end of the war he was in England engaged in the preparation of serums and vaccines. Previous to that time he worked in the Imperial Cancer Research Laboratories under Dr. Murray, although he is now working under a grant from the newly established National Institute for Medical Research. Altogether, I understand he has had fifteen years of good training in cancer research. It was Dr. Murray, himself, who called my attention to his work and sent me out to Mill Hill to see him. This place is on the outskirts of London about eight or ten miles north of Russell Square. In this connection I may quote a paragraph from Dr. Murray's Twenty-third Annual Report of the Imperial Cancer Research Fund, printed in November of this year and received by me to-day:

The essence of Dr. Gye's conception is that malignant growth results from the concurrence of two factors—an ultra-microscopic microbe and an unstable chemical factor derived in his experiments from propagated malignant tumours of animals, and it would be erroneous to regard either of these factors, singly, as the cause of cancer. The direct evidence of this dual origin of new growths has so far only been furnished for the Rous fowl sarcoma and for a transplantable sarcoma of the mouse. For other tumours the evidence is indirect, cultures of these supplying the ultra-microscopic microbe, the specific unstable chemical factor being supplied by an extract from the Rous fowl sarcoma, in which species of animal the two injected simultaneously gave rise to progressively growing sarcoma. Injected singly, they are inert. The delicate racial and tissue specificity governing the transmission of malignant new growths therefore attaches to the labile chemical factor, and not to the microbe. One of the gravest objections to previous forms of the parasitic hypothesis of cancer is thereby met. These experiments have only been possible by the use of the propagated animal tumours which are sufficiently exempt from ordinary bacterial contamination, and it is a legitimate source of satisfaction to this Fund that their prevision in maintaining this material over many years, including the war period, should have received its due acknowledgment and reward.

It is obvious that this dual conception of the etiology and pathology of cancer, should it be confirmed, introduces a new orientation in every aspect of the cancer problem: statistics, epidemiology, causation, prevention, and treatment. The new problems, being susceptible of direct experimental study, speculation and anticipation should be strongly deprecated.

The investigations of Mr. J. E. Barnard, F.R.S., are complementary to those of Dr. Gye, to whose deductions, however, they are not essential. . . .

Recently Dr. Carrel, of the Rockefeller Institute in New York, has produced an embryoma of fowls by injecting minced embryonic tissue, but here also a second factor was required. He used for this second factor a dilute solution of indol. This tumor has been transplanted, also with the help of indol, and now grows as a sarcoma, but he has been unable to cultivate any virus, it is said.

There was a very discouraged feeling among cancer specialists twenty years ago when I first began to be interested in tumors and to frequent the company of the oncologists. But this has now given place to eager interest on the part of many of the younger men. Important and far-reaching discoveries have been made and so many competent men are now at work on the various phases of the difficult problem that many more discoveries must soon follow. I am speaking to you only of one

branch of the great subject, namely, that which deals with causation. Unless I am far wrong, we shall know the cause of cancer (both sarcoma and carcinoma) beyond any doubt within a decade or two and perhaps very much sooner. The air is full of expectancy. Then the discovery of the cause of all the other tumors of obscure origin, such as glioma and the giant-cell bone tumors, must soon follow.

Warburg believes that anaerobic conditions are the explanation of tumor growth. According to his idea, our bodies and those of all animals are a mosaic of two kinds of cells, the great majority of the cells being aerobic. Under normal conditions the few anaerobic cells are controlled and kept in abeyance by the great mass of aerobic ones. Under certain abnormal conditions, such, for example, as the influx into an ulcer of aerobic organisms, avid of oxygen and the destruction of the aerating blood vessels, the aerobic cells are killed off and under cover the few anaerobic cells begin to grow rapidly as a tumor. Montrose T. Burrow's opinion[2] is that anything will cause cancer which leads to a stagnation in the tissues. Living cells excrete, he says, a substance which he calls *archusia.* This is the driving power—that which causes the cells to multiply. Ordinarily, it is abundant only in embryonic tissue and in the bodies of young animals. When normal growth is reached the blood circulation carries away all excess of it. Destruction of blood vessels and the attraction of body cells to a given center by bacteria, worms or non-living substances, tar, for example, or oil, with the resultant crowding cause this archusia to pile up locally inducing excessive uncontrollable growth and a tumor is the result. These are very seductive hypotheses and correspond very well to the fact that cancers never begin in a perfectly sound tissue. The origin of malignant tumors must be due either to some such train of phenomena as Warburg and Burrows have described, or else to the direct action of parasites. In

[2] See *Archives of Internal Medicine,* September 15, 1925.

either event it would seem as though multiplying extraneous organisms must be the activating thing in most cancers. McCarty, of the Mayo Laboratories, told me that he had examined more than one thousand breast cancers in sections under the microscope and that he had never found one which had not been preceded by the inflammatory condition known as mastitis. Such a theory as this would also account for the absence of the bacteria in all Blumenthal's transplanted tumors and for the fact that they have not been able to cultivate their organisms from human breast metastases or unruptured primary human tumors.

The next few years ought to throw much light into many of these dark places. I feel very hopeful for a relatively early solution of the whole terrible problem of cancer etiology, and then I believe a rational treatment will follow. Already in the selective action on cancer cells of certain chemicals, such as Kotzareff's radium emanation and Blair Bell's lead salts, we have the beginnings of a rational therapy.

Recently for the first time I have observed tumor-bearing cysts in connection with crown-gall. These I produced numerously in the pith of sunflower stems by inoculating the crowngall organism into young flower heads by means of needle pricks without injection. In that way I obtained cavities in the pith often extending over long distances, twelve to twenty inches or more. These cavities are lined by a fine-celled membrane covered with acicular and glandular soft white hairs, like those on the surface of the plant. They are subtended by vascular bundles, having always a reversed polarity. These abnormal xylem-phloem bundles are often fused into a compact cylinder surrounding the cavity and forming an elongated perfect stele in the center of the stem, outside of which in every direction are the normal pith cells. The tumors on the walls of these cysts are discrete or fused and frequently they are of considerable size, stretching and rupturing the cysts.

ON THE MIGRATION OF OVA FROM ONE UTERINE HORN TO THE OTHER IN THE ALBINO RAT; AND SOME EVIDENCE INDICATING A NEW OVARIAN HORMONE

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INTRODUCTION

THIS paper gives experimental evidence that in the albino rat there is no migration of ova from one horn of the uterus to the other after semi-spaying. It also presents certain facts which seem best interpreted as indicating the presence of a hitherto unknown ovarian hormone.

The junior author performed all the operations, observing ordinary aseptic precautions. The senior author is alone responsible for the statement of results here presented.

MATERIALS AND METHODS

The rats used in these experiments were excess animals from the control line of another experiment which the senior author has carried on for the past four years. These rats were taken from the control sixth, seventh and eighth generations of a brother-sister inbred line, this line originating from a single brother-sister mating in 1920. This pair of rats was itself derived from a long line of semi-inbreeding prior to 1920. The material is approaching the homozygous condition.

In the two experiments herein reported, the controls are in some cases brothers and sisters of those operated upon, in other cases the same individuals serve first as controls, later as experimental animals, and in all cases the relationship is of no greater degree than that of various kinds of cousinship within a strictly inbred line.

EXPERIMENT I

In this experiment twenty rats were semi-spayed; eleven on the right side and nine on the left. All the animals recovered quickly and completely from the effects of the operation. A few days later each was mated. Only vigorous young adults were used, and all the females were known not to be pregnant at the time of the operation.

As each female became pregnant, it was etherized and the body cavity opened. A record was made of the number and location of the developing embryos, and the uterine horn on the spayed side was examined to determine whether it was normal in appearance.

In every case the non-gravid horn was normal macroscopically.

Not a single embryo was found on the spayed side, while as many as nine in one case were crowded into the left horn. Cases of six, seven and eight embryos in one horn were fairly common.

Some of the rats were not opened until within a day or two of parturition. Those having a large number of embryos in one horn were not asymmetrical in external appearance and upon opening such females a beautiful adjustment of the viscera to the unusual condition was found.

Table I gives the data of this experiment and shows perfectly clean-cut results. Whether the litter be large or small, it is always implanted in the cornu on the side of the remaining ovary.

There is considerable discussion in the medical literature concerning the possibility of migration of ova. Of course this is not experimental and much of it may be uncritical. Nevertheless, several reported cases in which the ovary of one side and the oviduct of the other being removed, pregnancy resulted, indicate that migration across the body cavity did take place. This is called external migration to distinguish it from internal migration, which is within the uterus.

TABLE I

Right Ovary Removed			Left Ovary Removed		
Rat Number	No. Embryos left horn	No. Embryos right horn	Rat Number	No. Embryos right horn	No. Embryos left horn
1	3	0	16	7	0
2	7	0	17	6	0
3	2	0	18	6	0
4	8	0	19	7	0
5	5	0	20	8	0
6	9	0	21	6	0
7	5	0	23	2	0
9	3	0	24	9	0
13	7	0	28	6	0
14	2	0	......	......	......
15	6	0	......	......	......
Mean litter size, 5.70 ± 0.237					

Our work being experimental, only similar work will be considered in this discussion. The literature, so far as we are aware, consists of an abstract by Paul B. Kinney ('23), and several papers by George W. Corner ('21a, '21b, '22).

Kinney ('23) states his entire results in the following brief paragraph:

> One ovary was removed in a series of twenty-five guinea-pigs and the pigs mated upon recovery. No implantation was found upon the operated side, although every care was taken to use only perfectly normal animals and although recovery from the operation was rapid, uncomplicated, and the uterus on the operated side was found practically undisturbed at necropsy.

It is needless to point out that these results are in entire agreement with those on rats as shown in Table I.

Corner ('21a, '21b, '22) worked with swine. His methods were entirely different from ours. Pregnant uteri, with ovaries attached, were secured from packing houses. A count was made of the corpora lutea of pregnancy in each ovary and the number of developing embryos in the corresponding horn noted.

The only objection to this method is that it does not take into account a possible early prenatal mortality. However, the tables given by Corner show so clearly that

internal migration of ova does take place in swine that the above-mentioned objection seems ill founded.

From several of Corner's tables I have collected a few of the clearest and most extreme examples of migration and constructed the following table:

TABLE II

MIGRATION OF OVA IN SWINE

(from Corner's tables)

Corpora lutea left ovary	Corpora lutea right ovary	No. Embryos left cornu	No. Embryos right cornu
8	0	4	4
6	4	5	5
5	1	3	3
8	2	5	5
8	0	4	4
5	1	3	3
5	0	3	2
2	7	5	4
8	1	4	5
11	0	8	3

Table II is quite convincing of the fact of migration in swine. The question now arises, Why these discrepancies between rats and guinea-pigs on the one hand and swine on the other?

In the first place it must not be forgotten that in swine both ovaries were intact, *i.e.,* conditions were normal. In the semi-spayed rats any hormone relation between ovary and cornu is destroyed by the removal of the ovary.

Frankel ('03) and Marshall and Jolly ('05) claim in the rabbit and rat the presence of the ovary is essential for the implantation and early nutrition of the embryo. If this be true, then migration of ova might have taken place in our animals, but failing to implant, perished. This is a plausible explanation and seems to reconcile the different results. There are, however, some facts in this present instance which seem to render it invalid.

Litter size is being studied in the stock from which our animals came, and we have a great deal of data on the

subject. That which is pertinent for this discussion is shown in Table III.

TABLE III

Generation	Mean litter size
6	5.65 ± 0.29
7	5.31 ± 0.22
8	6.38 ± 0.18
Semi-spayed	5.70 ± 0.24
Oviduct excised	3.80 ± 0.28

It will be recalled that our animals were taken from generations six, seven and eight of an inbred line. Table III shows the mean litter size of the rats in these three generations and also that of the semi-spayed rats. All the semi-spayed rats themselves contributed to or are a part of the litter size data of generations six, seven and eight.

It is possible then to make a very direct comparison between the litter size of the semi-spayed rats and that of the three generations from which they were taken. When this is done it is apparent that the difference is not significant. The semi-spayed litter size of 5.70 is slightly larger than that of generations six and seven, slightly smaller than that of generation eight, *i.e.,* it falls approximately midway between the range of variation shown by the three generations.

The point, then, is this, that if any considerable number of ova, or even a very small number, migrate in rats only to die through failure to implant, the effect on the litter size would be apparent in as carefully controlled material as this. This, however, is not the case. The litter size measures up well and even exceeds that of two of the three generations proper for comparison.

These results seem to indicate that in semi-spayed rats all the embryos develop in the cornu of the unoperated side, and, further, the litter size data seems to preclude the possibility of ova migrating to the other horn and

dying through failure to implant. This latter point would not be insisted upon if our material were not so nearly homozygous and the control generations and semi-spayed groups so closely related, indeed, in many cases being the same animals.

EXPERIMENT II.

Semi-spayed rats produce just as large litters as normal rats (Table III). In experiment II an attempt was made to discover the factor or factors responsible for this. Since this greatly increased activity of one ovary occurs only in the absence of the other, suspicion was attached to the ovary as the seat of the physiological factors at work.

Before proceeding with the results of our experiment, there are two papers in the literature to which reference should be made.

Carmichael and Marshall ('07–8) semi-spayed rabbits and found a compensatory growth of the remaining ovary. In some cases the hypertrophy was 100 per cent., in other cases not so marked; but in one rabbit where one ovary was removed and also half the other, the hypertrophy of the remaining ovary was very great, leading the authors to say that the greater the amount of ovary removed, the greater the power of hypertrophy.

Hatai ('13) studied the effect of semi-spaying on the weight of the remaining ovary. He found that "in the semi-spayed series the compensatory growth of the remaining ovary is almost perfect and the single ovary has nearly twice its normal weight." He then goes on to say, "Exactly what structures are responsible for such a compensatory growth of the ovary still requires to be investigated." It is believed that the evidence about to be presented may throw some light on why compensatory growth in the ovary occurs.

After semi-spaying the remaining ovary grows to approximately twice its normal size and performs approximately double the amount of work that it does under nor-

mal conditions. Our next experiment was to excise a piece of either the right or the left oviduct of fourteen females, leaving the ovary intact and not disturbing its blood supply. The tube was tied off in two places with surgical silk and a part of the tube was excised between the ties. With the tube cut and both cut ends tied off it was impossible for any eggs to reach the uterus, yet any ovarian hormones could be emptied as freely into the blood stream as before the operation.

After recovery from the operation, which was rapid and complete, the females were mated. In the following few months they produced a total of forty-five litters. Table IV gives the data for litter size when one ovary only is functional in reproduction, while the other is present and normal in every respect except that its eggs are unable to reach the uterus.

TABLE IV

A TABLE SHOWING LITTER SIZE OF INBRED ALBINO RATS WHEN ONE OVARY IS TIED OFF FROM UTERUS, BUT OTHERWISE UNDISTURBED

Rat number	Right ovary tied off	Rat number	Left ovary tied off
469	6, 4, 6, 4, 5	477	4, 4
467	5, 3, 2	277	2
236	3, 3, 4, 3	234	1, 3
237	4, 5, 4	357	4, 1, 2, 2, 6
268	3, 7, 6	456	4, 3, 5
358	7, 6, 7, 3	347	3, 4, 7
459	2, 3	....	....
337	3, 5, 3, 2, 4	....	....

Mean litter size, 3.80 ± 0.156

The mean litter size is 3.80 ± 0.156 when both ovaries are present, but only one is responsible for the litter. When one ovary is responsible for the litter, the other ovary being removed the litter size jumps to 5.70 ± 0.237. It seems that in the first case we are getting half litters and in the second instance whole litters. A statement of

the difference between the litter sizes under the two conditions is as follows:

$$5.70 \pm 0.237 - 3.80 \pm 0.156 = 1.90 \pm 0.29.$$

Since this difference is over six times its P.E., the odds against the chance occurrence of a deviation as great as this is something more than 19,300 to 1.

The explanation of these results tentatively suggested is that between the two ovaries there is a reciprocal hormone relationship, each inhibiting the other from growing beyond a certain size or producing over a certain average number of ova per ovulation. In semi-spaying the remaining ovary is released from the inhibiting action of the other and hence hypertrophy and full size litters. When one ovary is tied off it can not participate in reproduction, but as an inhibitor it is fully functional, hence the half-size litters.

Since the above was written two papers bearing on this subject have appeared. One is by Hartman ('25) on the functional compensatory hypertrophy of the opossum ovary, and the other by Allen, Kountz and Francis ('25) on selective elimination of ova in the adult ovary. These latter authors attribute the functional hypertrophy of the remaining ovary after semi-spaying to the increased amount of nutriment available, *i.e.*, when one ovary is removed, the utilization of its share of reproductive nutriment by the remaining ovary lessens the severity of selective elimination and allows the maturation of nearly twice the number of ova which would normally have been produced in this ovary.

While opportunity is lacking in this note for a discussion of the "selective elimination theory" of Allen, it may be remarked that survival is not based primarily on a struggle for food, but is an adjustment to environment. Can it be said that there is adjustment to environment within an ovary?

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AN ANALYSIS OF SOME FUNCTIONS AND ATTRIBUTES OF THE INDIVIDUAL[1]

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A GENERATION ago most of the bodily functions and attributes of all living things were interpreted as qualities of the individual as a whole. Life was considered as something imposed upon certain forms of substance as an attribute of those masses of matter to which we apply the name of an individual. Disease, normal bodily functions and the numerous characteristics of the individual were construed as aspects of the whole being of the organism.

Less than a century ago a new concept of structure and function was injected into the biological sciences. As a consequence of this innovation, there has been a complete revolution of the entire method of thought in the consideration of problems pertaining to the body. I refer to the pronouncement of the cell theory which, in its simplest form, merely postulates that structural units termed cells furnish the basis for all structure in living organisms. Beyond this, however, it has come to be recognized that in these structural units are to be found the ultimate explanations of all bodily functions, both normal and abnormal. It is due to the study of these units that so much progress has been made toward an explanation of vital phenomena in terms of purely physical and chemical laws.

In rather brief manner, I would like to point out some lines of recent advance in the understanding of the organism in terms of these cellular units. For this purpose I have chosen a series of topics which, in themselves, seem to lack coherence, but for evidence of their unity demand nothing beyond the citation of their intimate correlation

[1] Address of retiring president of Illinois Chapter of Sigma Xi.

in the economy of the living being. We shall direct our attention to some of the problems pertaining to death; to its antithesis, bodily repair; and to the theme of cell numbers with its corollary, body size.

I

Death is a phenomenon which to most of us is absolute—permitting of no evaluation in comparative degrees. Yet, in the light of modern concepts of living matter, we must admit that death is one of the things which makes life possible. In this paper, I do not intend to go into the philosophical aspects of the ultimate nature of life and of death, but would like to point out some of the biological interpretations and conclusions to be derived from a consideration of these topics.

Of the numerous attributes of living organisms, no one is more significant than that of the continuous physical and chemical changes which characterize animate matter. This fact has become so generally admitted that it is accepted as little short of a truism that matter which has reached a state of stability in its composition can not exhibit the attributes of a living organic being. Protoplasm, the physical basis of life, is a highly complex mixture of chemical substances in a colloidal system. Life is manifest in such a system only as an accompaniment of continuous reaction and interaction between the substances of the colloidal system. In truth, some physiologists have defined life in terms of the reaction and interaction between certain protein molecules and the medium in which they lie. Essentially, these reactions result in the breaking down of organic matter into dead waste substances, with an attendant liberation of energy which becomes manifest in the movement, light, heat or other attributes or activities of the living organism. Thus the parts perish that the whole may live. Death is so intimately a function of life that the cryptic statement of the losing of life in order to gain it bears a biological significance far beyond any that could have been ascribed

before our present-day understanding of the physico-chemical nature of living matter.

In our popular thinking, we seem to have come to the point of considering death as merely the cessation of the individual, something at the end of life, apart from it and an experience previously unknown to the animate masses of matter which it visits but once. In this light, death is an imposed phenomenon, apart from life and marking the close of the cycle of development. This cycle we normally recognize as coursing through successive epochs, starting with birth and in sequence running through youth, maturity and senescence and ultimately ceasing with death and dissolution. However, the continuous dissolution of the integral parts of the living organism demonstrates that death is not an innovation imposed at the close of the life cycle, but the same phenomenon is present at the very inception of life in the first beginnings as a single cell, the fertilized egg. The initiation of development is in no small measure due to the introduction of death bringing processes into the protoplasm or at least to the hastening of those already resident therein. Throughout the early development of the individual and on through the periods of youth and maturity the continuous disintegration of its parts is immediately compensated by other processes, just as characteristic of living matter, which result in the creation of new living matter to replace that which has ceased to live. This catalytic power of protoplasm permits non-living food material in the presence of living protoplasm to become endowed with the properties of life and transmuted from the realm of inert matter into the kingdom of the living things. Not only is compensation for the losses due to the destructive processes of life met in this manner, but through the early periods of the life cycle these constructive processes proceed at a rate in excess of the destructive phenomena with the consequence that growth takes place. Yet all this is within a system which is made possible only through the operation of destructive, death-bringing processes.

II

Senescence, with its accompanying changes in the structure and functions of the body, marks the ascendence of the destructive forces within the living protoplasm. It is interesting to note that in the early works on senescence this condition was thought to have some connection with the increase in age of the individual without any adequate conception of the intimate changes involved. It is only within recent times that cytological evidences have been sought and found. Thus, it has been determined that the individual grows old because the cells of which the body is composed have grown old. Needless to say, this process of ageing is not observable in equivalent degree in all parts of the body.

Some of the most profound changes marking senescence are to be found in the various glands and in the nervous system. It has been shown, for example, that with increasing age the cells of the central nervous system undergo conspicuous modifications. There is a progressive alteration of the ratio between volume of the nuclei and that of the surrounding cytoplasm. Since it is generally conceded that the nucleus controls much of the activity of the cell, this altered ratio between nucleus and cytoplasm is accompanied by modifications in the activity of the nervous system. In a newly emerged honey-bee, the nucleus of the ganglion cells is so large that the surrounding cytoplasm is reduced to a thin envelope enclosing the nucleus. In the aged bee, by way of contrast, the outline of the entire ganglion cell retains essentially the same size, but a minute nucleus rests in the center of each large mass of cytoplasm. This is a type of cellular modification accompanying increased age that has become recognized as characteristic of the senescent stage.

Similar studies have revealed a comparable condition in the brain of man. Before birth or in the new-born infant the nuclei of the brain cells are relatively enormous in size. Throughout life there is a progressive alteration of the ratio existing between the volumes of

the nuclei and their surrounding cytoplasm. In an aged man who has reached the senescent state, the nuclei have dwindled to but a small fraction of their initial size.

The really significant fact concerning this series of progressive changes in the brain cells consists in that the brain cells of the higher animals possess little or no capacity for replacement. No one has ever detected evidence of a ganglion cell in the process of multiplication. In this respect, nerve cells pertain to that category of tissues which lack the powers of restorative regeneration. Under normal functioning, the individual cells of the brain experience the continuous cycle of internal changes and repair incident to life, but possess no powers of repair beyond the slight compensatory restitutions coexistent with life. Unlike the skin, the liver or numerous of the other bodily organs, the brain cells have lost the powers of substituting new individual cells or groups of cells for those that have been destroyed through injury or through accident. Roughly speaking, injury to the cells of the nervous system may be classified either as direct physical violence by some external instrument or agency, or injury arising from the normal functioning of the brain cells. The latter of these presents numerous problems of absorbing interest for the investigator.

It has frequently been a matter of observation that experimental animals subjected to complete exhaustion display profound changes in many of the glands and in cells of the nervous system. If such stimulation is not carried too far, the individual cells may ultimately recover, but extreme cases involve the ultimate complete disappearance and loss of the nuclei from certain types of ganglion cells accompanying death resulting from shock. Thus, in man as well as in experimental animals, certain types of exhaustion and shock produce death with no apparent external cause. Examination of the brain of an organism killed in this manner reveals regions wherein the nuclei of the ganglion cells have completely disappeared. Death in such an instance seems to be due

to disintegration of the nervous system. This fact with many of the applications growing out of it is one of the contributions to science growing out of studies made possible and necessary in the course of the great war.

The unaccountable lack of power of recovery of the damaged nerve cells under shock and exhaustion marks the tissue of this important system as radically different from the majority of tissues in the human body. Most cells have the power of repair so highly perfected that any loss even of groups of cells from a tissue is compensated by a replacement which is commonly designated as the power of regeneration. In the broad use of this term, it is applied to all categories of renewal, ranging from the replacement of a portion of a single cell to the restoration of the entire body from a small fragment as in the case of small pieces of a mutilated Hydra or planarian giving rise to an entire individual. Some writers have chosen to differentiate between these two extremes, designating the former as physiological and the latter as restorative regeneration. Physiological regeneration pertains primarily to the substitution of new atoms and molecules, brought into the body as food, to replace similar units broken down in the performance of normal bodily functions. In contrast, restorative regeneration involves replacement, frequently of more conspicuous nature, as in the regeneration of the leg of a salamander or the tail of a fish.

Typically, restorative regeneration in the Metazoa, or higher animals, is possible only through the increase in numbers of cells. But some sorts of tissues have recently been shown to lack the power of multiplying the cells of which they are composed after the cells have once become specialized and therefore regeneration is impossible. In the human brain, for example, there is practically conclusive evidence, as already cited, that injury involving the destruction of the nerve cells is never repaired and if general enough produces death of the individual.

III

Students of the cell have only recently begun to appreciate this fact, for there has been a general belief that continued power of producing like cells is an essential characteristic of all living tissues. In a considerable number of different kinds of animals the entire body, excepting the reproductive cells, is made up of cells which have lost all further powers of increasing their numbers. As a consequence, beyond the period of embryonic development in such forms the number of cells within the entire body is fixed throughout the life of the individual. In many of these forms, not only is the number of cells fixed for the individual, but through some intricate manipulation of the mechanics of heredity every individual of the same species possesses the identical number of cells. This condition, which in the literature has been designated as cell constancy or as nuclear constancy, has many important contacts with the general problems in biology. Not the least of these is the problem of relative size of the organism.

Differences in metabolism, dissimilarity in habits, diversity in structure, inequality in heredity and even variability in the physical surroundings all go toward a full explanation of size differences in animals. From time to time, limited observations have furnished the basis for broad generalizations as to the principles underlying body size. By far the greater number of biologists who have investigated this problem have been advocates of the premise that body size is more or less directly dependent upon the numbers of cells present in the body. In reaching this conclusion, the evidence available from numerous sources throughout the animal kingdom has been ignored, for organisms representing many groups display fixed numbers of cells regardless of the individual diversity in size. In some instances, even the identical spatial relations of the individual cells are maintained. But not all organisms maintain a constancy in the number and arrangement of their cells. Here again we encounter an illustration of the futility of attempting to

subsume under one category all the diversified aspects of nature. We have in body size a series of conditions which are not expressible in terms of a single law. Size in some animals is a function of cell numbers, while in others it is just as clearly a function of cell size.

Contemplation of the mechanism which renders such a fixed formula of bodily structure possible awakens a feeling of bewilderment. It has long been known that the normal early development of many organisms follows rigidly established lines. In the past generation, much attention was directed to the problems of cell lineage, wherein the orderly processes of development follow one another in such perfect sequence that it is possible to trace the history of given cells in early ontogeny and to predict what organs or parts of the adult are to be formed from them. Rigorous establishment of a condition of this sort can have but one underlying cause. Environmental factors are subject to too many vicissitudes to control development with such predictable nicety. Only through the utilization of conditions resident within the fertilized egg or developing therein can such intimate direction of the path of development be accomplished. The conditions which make cell lineage possible, extended, result in producing cell constancy. Here we gain an intimate glimpse of the intricacy of the mechanism of heredity. It is ordinarily acknowledged that some mechanism within the egg determines the sundry bodily characteristics and mental attributes of the individual. In cell lineage and in cell constancy there seem to be just as strong evidences that there is a hereditary predetermination of the total number and sequence of the cell divisions requisite to produce the cells which go to make up the body.

IV

The question might well be raised as to the broader significance of some of these problems. The topic of cell numbers and that of ability of self-repair have many contacts with the field of human biology. The whole

problem of education centers around the elaboration and coordination of the tissue which we speak of as the nervous system. Yet the cells of which the brain, and other parts of the nervous system in man, is composed are laid down very early in the course of development of the individual, even before birth, and so far as direct evidence goes then lose all powers of further increase in numbers. Physical limitations to methods of examination of an organ the size of the human brain have precluded the possibility of determining whether the number of cells is constant for the species. Aside from this point, there seems to be definite assurance that power of self-perpetuation is lost to cells of the nervous system. In this light, we have a possible means of understanding why education seems to be limited to the possibility of developing the abilities rather than creating new ones. The nerve cells with which the individual is endowed at birth seem to provide the background for mental growth.

Upon first glance it seems a far step from the consideration of cell numbers in organisms to the cancer problem. Yet, biologically, the two problems have many points in common. Cancerous growths are characterized by a riotous career of reproduction in certain types of cells. When students of cell mechanics discover either the cause underlying stimulation of cells to continued division or a means of inhibiting the reproduction of cells, in either instance there lies the possibility of application of cell responses to the control of cancer.

If I have succeeded in presenting some of the evidences of the biological aspects of death as a consequence of agencies resident within the living organism, and if I have made clear some of the relations of growth and cell numbers to the problems of body size, I have accomplished the purpose with which I began. Suffice it to say that in spite of the progress that has been made toward understanding the individual in terms of its parts there yet remains much that is untouched.

SCIENCE AND SCIENTIFIC NAMES

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There are now some 160,000 names of zoological genera, of which possibly 20,000 are synonyms or homonyms, and to this list there are being added each year 1,000 to 2,000 names of new genera, the average probably being about 1,500. Current zoological practice prohibits the duplication of generic names throughout the entire series and as a consequence various scientists have adopted prefixes, suffixes and other devices in a more or less successful attempt to obviate the creation of homonyms. A brief survey reveals an extremely interesting condition. Lists to the end of 1923 show nearly 3,500 zoological genera bearing the prefix Eu, over 2,000 each with the prefixes Para and Pseud, over 600 with the prefix Hemi, over 500 each with the prefixes Steno and Syn, over 400 with the prefix Acanth, and Acro, Isch and Pleur each with some 200 to 300 genera. Turning to the Insecta, we find in the Coccidae 99 genera with the root aspis and in the Itonididae or Cecidomyidae 100 genera with Diplosis compounds. A list of mammalian genera shows 348 with the root Mys and 268 with the combination Nycteris. There are surely plenty of mice in the field and possibly a superabundance of bats in the belfry. It should be noted in passing that these names, prefixes and roots have given very little aid in finding the taxonomic position of the genera, although with the exception of Eu they have four to eight letters each.

Naturally it becomes more difficult to find satisfactory combinations as the number of generic terms increases and this is suggested by some of the extremely long names, such as Asmithwoodwardia, Austroternoplatys, Brachygnathosuchus, Edvardotrouessartia, Hetaeroceocephalus, Proanthropomorphus and Pseudotarsone-

moides, to cite only a few cases. In addition, there are also nonsense names.

It is granted that generic names should express something characteristic of the genus. The wholesale use of prefixes and suffixes, such as those indicated above, and the increasing tendency toward extremely long names, 18 to 21 letter combinations, is very suggestive as to future conditions. It seems probable that some of these combinations have been used more for the purpose of avoiding homonyms than to express a characteristic of the organism. We have simply been adding syllables or groups of letters with little regard for consequences, although there have been a number of attempts to introduce more or less desirable changes. One of the earliest was that of Prof. Harting[1] in 1871, a system of class suffixes combined with ordinal prefixes. The use of letter formulae for kingdom, phylum, class, order and genus and numerals for species was proposed by Tornier[2] in 1898 for both plants and animals. The use of prefixes and suffixes for the ready placing of generic names of animals and plants was proposed by Herrera[3] in 1899, and a series of initial letters for classes and ordinal prefixes by Rhumbler[4] in 1910.

The next contribution appears to be by Jonathan Dwight, Jr.,[5] in which he makes a plea for a more logical nomenclature. This was followed by Prof. James G. Needham,[6] in which he proposes the use of older generic names in approximately their original significance and the designation of species by numerals for the use of general naturalists in particular. Those interested in nomenclature and the psychology of naturalists should read the various articles on this general subject which

[1] *Archiv f. Naturgesch.*, 4: 26–41, 1871.

[2] *Zoologischer Anzeiger*, 21: 575–580, 1898.

[3] *Science*, 10: 120–121, 1899.

[4] *Zoologischer Anzeiger*, 36: 453–471, 1910.

[5] *Science*, 30: 526–527, 1909.

[6] *Science*, 32: 295–300, 1910.

appeared shortly thereafter.[7] Dr. Heikertinger outlined another plan[8] in 1916, elaborating certain aspects[9] in a later paper.

The radical proposals to indicate taxonomic relationships and other data by prefixes, suffixes and other modifications of generic names appear to have failed on account of inherent weaknesses. It is probable that zoologists as a whole are opposed to modifications of generic names, even were this permissible under present rules, and especially if the change makes it very difficult, if not impossible, to connect the original name with the new term. The unsystematic use of prefixes, either initial letters or syllables of classes or orders, adds considerably to the burden of nomenclature without any very material advantage. Consequently, earlier proposals have not met with general approval and in the meantime we are progressing rapidly and creating numerous unwieldy names characteristic of the genus to only a slight extent and significant largely because of their place in our taxonomic system. Numerals, especially decimals, afford a ready means of grouping in a fixed or well-established classification. They do not lend themselves readily to the numerous transpositions in zoology. It is physically impossible for a naturalist to keep even a reasonable proportion of the 140,000 more or less current generic names in mind and reasonably well placed in the system.

In view of the above, it is suggested that the use of prefixes based primarily upon initial letters for the principal groups affords a way out of a taxonomic tangle, since these could be used without affecting in any material way the thousands of generic concepts and yet make it extremely easy to arrange these names in approximately their systematic position by the simple process of alphabeting. Each name would automatically,

[7] Cockerell, T. D. A., *Science*, 32: 428–429, 1910; Needham, J. G., *Science*, 32: 795–796, 1910; Ward, H. B., *Science*, 33: 25–29, 1911. Jordan, D. S., *Science*, 33: 370–373, 1911; Needham, J. G., *Science*, 33: 813–816, 1911; Girault, A. A., *Science*, 33: 373–374, 1911.

[8] *Zoologischer Anzeiger*, 47: 198–208, 1916.

[9] *Zoologischer Anzeiger*, 50: 41–54, 299–302, 1918–1919.

moides, to cite only a few cases. In addition, there are also nonsense names.

It is granted that generic names should express something characteristic of the genus. The wholesale use of prefixes and suffixes, such as those indicated above, and the increasing tendency toward extremely long names, 18 to 21 letter combinations, is very suggestive as to future conditions. It seems probable that some of these combinations have been used more for the purpose of avoiding homonyms than to express a characteristic of the organism. We have simply been adding syllables or groups of letters with little regard for consequences, although there have been a number of attempts to introduce more or less desirable changes. One of the earliest was that of Prof. Harting[1] in 1871, a system of class suffixes combined with ordinal prefixes. The use of letter formulae for kingdom, phylum, class, order and genus and numerals for species was proposed by Tornier[2] in 1898 for both plants and animals. The use of prefixes and suffixes for the ready placing of generic names of animals and plants was proposed by Herrera[3] in 1899, and a series of initial letters for classes and ordinal prefixes by Rhumbler[4] in 1910.

The next contribution appears to be by Jonathan Dwight, Jr.,[5] in which he makes a plea for a more logical nomenclature. This was followed by Prof. James G. Needham,[6] in which he proposes the use of older generic names in approximately their original significance and the designation of species by numerals for the use of general naturalists in particular. Those interested in nomenclature and the psychology of naturalists should read the various articles on this general subject which

[1] *Archiv f. Naturgesch.*, 4: 26–41, 1871.
[2] *Zoologischer Anzeiger*, 21: 575–580, 1898.
[3] *Science*, 10: 120–121, 1899.
[4] *Zoologischer Anzeiger*, 36: 453–471, 1910.
[5] *Science*, 30: 526–527, 1909.
[6] *Science*, 32: 295–300, 1910.

appeared shortly thereafter.[7] Dr. Heikertinger outlined another plan[8] in 1916, elaborating certain aspects[9] in a later paper.

The radical proposals to indicate taxonomic relationships and other data by prefixes, suffixes and other modifications of generic names appear to have failed on account of inherent weaknesses. It is probable that zoologists as a whole are opposed to modifications of generic names, even were this permissible under present rules, and especially if the change makes it very difficult, if not impossible, to connect the original name with the new term. The unsystematic use of prefixes, either initial letters or syllables of classes or orders, adds considerably to the burden of nomenclature without any very material advantage. Consequently, earlier proposals have not met with general approval and in the meantime we are progressing rapidly and creating numerous unwieldy names characteristic of the genus to only a slight extent and significant largely because of their place in our taxonomic system. Numerals, especially decimals, afford a ready means of grouping in a fixed or well-established classification. They do not lend themselves readily to the numerous transpositions in zoology. It is physically impossible for a naturalist to keep even a reasonable proportion of the 140,000 more or less current generic names in mind and reasonably well placed in the system.

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[8] *Zoologischer Anzeiger*, 47: 198–208, 1916.

[9] *Zoologischer Anzeiger*, 50: 41–54, 299–302, 1918–1919.

through the prefix, place itself without the labor of referring to a number of standard lists.

CODE PREFIXES FOR ZOOLOGICAL NAMES

These indicate a major group, phylum or class, order and family.

The first letter indicates the major group, phylum or class, the second the order and the third and fourth the families.

The first two are usually the initial letters of the major groups, the third and fourth letters are arbitrarily assigned and indicate the family position in some recognized arrangement, they being distributed somewhat through the alphabet in order to permit ready interpolations, should these latter prove desirable. Only pronounceable combinations have been used.

Prefixes are written as a part of the generic name except that the initial letter of the genus is also capitalized.

The prefixes automatically place each genus in its systematic position and in alphabeting bring into one series all genera of a family.

The prefixes add nothing to the definition of the genera other than that implied by their taxonomic position. They are simply a visible sign of generic concepts hitherto assumed except in systematic treatises and lists.

Prefixes do not alter in the slightest degree the nomenclatural status of the genus so far as date of erection, description, type and author are concerned.

They create no impassable break between present nomenclature and that suggested, since the capitalization of the original genus makes it easy to recognize the unmodified name for those who prefer the present system.

Prefixes may function not only as a classifying guide but may also be used to differentiate between many desirable generic names now unavailable because they have been used in some other class of the animal kingdom. The essential in zoological nomenclature is to prevent duplication and therefore undesirable confusion in genera, and it is held that the use of prefixes as valid,

differentiating criteria in zoological genera would liberate thousands of useful descriptive terms which could be duplicated in the various classes, at least, without the slightest danger of confusion and without invalidating in any material way earlier work, provided these compounds have no retroactive effect.

Transfer from one family to another or to another order or class would be made under current nomenclatural rules as heretofore, except that the prefix would be changed to accord with the new position. In other words, the prefixes would be permanent only so long as the genus remained in a designated group.

Generic names would be proposed as heretofore and convey some idea of the genus, the prefixes being determined by their taxonomic position.

The assignments of letters or groups of letters to the various zoological groups should not be construed as attempting to establish any special classification. It is necessary to adopt some guide if a comprehensive system is proposed. There is nothing in these prefixes which would necessitate the arrangement of phyla, classes or families in any definite order, yet if they are used as a basis for alphabeting, especially in large general works, the genera will inevitably fall in related groups.

The combinations are unfamiliar and on this account appear difficult. Workers in various groups will quickly learn their significance and soon use them with facility.

The early adoption of a system of class prefixes would have made possible immense series of characteristic generic names of reasonable length and our present literature would not be burdened with unreasonably long, sometimes nonsensical and frequently far from characteristic generic designations.

The adoption of a system such as is proposed would greatly simplify the selection of generic terms in the future and should result in a much more logical nomenclature.

The application of this system is suggested by the following:

TENTATIVE SKELETON CONSPECTUS OF CODE PREFIXES FOR ZOOLOGICAL NAMES

Phyla: O, Protozoa; S, Porifera or Spongiae; C, Coelenterata; E, Echinodermata; V, Vermes; F, Brachiopoda; N, Bryozoa; D, Mollusca.

Classes: K, Crustacea; U, Arachnida; I, Insecta; G, Prochordata; P, Pisces; R, Reptilia; B, Batrachia; A, Aves; M, Mammalia.

Ob: class, Heliozoa; phylum, Protozoa.
  Obac: order, Aphrothoraca; phylum, Protozoa; class, Heliozoa.
Sa: class, Calcarea; phylum, Porifera.
  Sade: order, Homocoela; class, Calcerea; phylum, Porifera.
Ca: class, Hydromedusae; phylum, Coelenterata.
  Cade: order, Anthomedusae; class, Hydromedusae.
Ic: order, Coleoptera; class, Insecta.
  Icba: family, Cicindelidae; order, Coleoptera.
    Genera with prefixes: IcbaAmblycheila, IcbaOmus, IcbaTetracha.
  Icbo: family, Haliplidae; order, Coleoptera.
    Genera with prefixes: IcboBrychius, IcboHaliplus, IcboPeltodytes.
  Icro: family, Coccinellidae; order, Coleoptera.
    Genera with prefixes: IcroHyperaspis, IcroHelesius, IcroHyperaspidius.
Il: order, Lepidoptera; class, Insecta.
  Ilaf: family, Hepialidae; order, Lepidoptera.
    Genera with prefixes: IlafStenophis, IlafHepialus.
  Ilke: family, Cossidae; order, Lepidoptera.
    Genera with prefixes: IlkeGivira, IlkeAcossus, IlkePrionoxystus.

The alphabeting possibilities of prefixes are illustrated by the following list of Coleopterous genera with tentative prefixes:

IcabMaronetus	IcakLudius
IcabNomaretus	IcakMeristhus
IcabPemphus	IcarCathartus
IcabScaphinotus	IcarOryzaephilus
IcaeAgyrtes	IcarPediacus
IcaeLyrosoma	IcarSilvanus
IcaeNecrophorus	IcleAgaeocera
IcaeSilpha	IcleDystaxia
IcafNeomedon	IclePtosima
IcafOligopterus	IcleTyndaris
IcafPaederus	IctuAulacoscelis
IcafStenus	IctuDonacia
IcakAeolus	IctuGalerucella
IcakAgrypnus	IctuHaemonia

The new code prefix has been applied tentatively to the Coleoptera, Lepidoptera and Diptera and by the use of simple compounds such as those indicated above the

family position of every genus has been indicated. The four letter combination makes it possible in the lower, less known phyla or large groups to at least place the genera in their orders and in some cases at least in the families. Utility has been a major consideration in assigning the prefixes, even though it involves some inconsistency. Nomenclature should possess a certain adaptability to the requirements of various groups. It is proposed to use all genera with appropriate prefixes, as written above, in literature just as we are using genera to-day. It should be remembered that all these letter assignments are purely tentative. A better method may be devised. Should the system receive favorable consideration from many naturalists, the authors would gladly work out, in cooperation with authorities in the various groups, a comprehensive system which can hardly fail to better existing conditions materially. Constructive criticism is welcomed and that of another nature may be of value. We are concerned mostly in bettering nomenclatural conditions. A modification such as suggested above will not better matters greatly in the next few years. The full benefits will hardly be apparent for some time. The system adds to the length of generic terms and to this extent is objectionable. It may be possible in the future to eliminate some of the nearly 2,000 prefixes each of Para and Pseud, in favor of more definitive prefixes and thus bring many generic names within reasonable limits, and that without changing the zoological status of the genus or materially affecting the significance of the original term. A system of alphabeted prefixes agreeing with the main outlines of recognized classifications in phyla and classes would do much to stabilize nomenclature without offering serious obstacles to advisable changes.

SHORTER ARTICLES AND DISCUSSION

THE OCCURRENCE OF PIGMENTED FACETS IN WHITE EYES IN DROSOPHILA MELANOGASTER

During the course of a series of experiments involving the securing of permutations of mutant eye colors of ***Drosophila*** *melanogaster* a stock of purple white flies was established. As was to be expected from results secured by Morgan and Bridges (1), the color of the eye in this permutation was white and not distinguishable from the eye color of the fly containing only the white mutation.

However, on January 24, 1924, while examining flies from culture bottle 10 of this stock under the low power of the compound microscope ($\times 100$) in order to determine whether any slight differences between these and the ordinary white flies might exist, a female was found in which a facet near the posterior dorsal margin of the right eye was distinctly pigmented. A careful study of this facet under the high power ($\times 430$) with varying intensities of light showed the pigment to be red and as nearly as could be determined deep within the ommatidium. The rest of the eye was white. This female died without reproducing, but the flies of this stock were carefully examined for a reoccurrence of colored facets. Each fly, while etherized, was placed in four different positions so that all the facets of both eyes could be examined under the compound microscope. An individual record was kept of every fly examined.

On February 2, in examining flies from stock culture 20 made up on January 21 from a single mating from stock culture 10, three flies were found with one facet of the left eye pigmented, four flies with one facet of the right eye pigmented, and one fly with two facets of the right eye and one of the left eye pigmented. A sketch of the eye with the position of the pigmented facet was placed in the record and each fly was put in a separate vial and numbered so that matings could later be made up.

From this stock 104 flies were examined, in which 40 per cent. were found to have one or more facets of one or both eyes pigmented. Stocks 21 and 23 made up at the same time as stock 20 showed pigmentation in 31 per cent. and 50 per cent. of the flies examined. Fifteen third generation matings were made up from flies having pigmented facets from bottle 20 and of these

TABLE SHOWING THE OCCURRENCE OF PIGMENTED FACETS IN FOUR GENERATIONS OF WHITE-EYED DROSOPHILA

No. of generation	No. of stock, etc.	No pigment in either eye	Pigment in one facet of left eye	Pigment in one facet of right eye	Pigment in one facet of both right and left eye	Pigment in several facets of left eye	Pigment in several facets of right eye	Pigment in several facets of both eyes	Pigment in many facets of one eye	Pigment in many facets of both eyes	Per cent. of eyes pigmented
I	Stock 10.	?	?	1	?	?	?	?	?	?	?
II	Stock 20.	62	14	17	3	3	2	2	0	1	40%
II	Stock 21.	25	3	3	0	1	3	0	0	1	31%
II	Stock 23.	9	0	6	0	3	2	2	0	0	59%
III	Stock 30. ♀ 1 Facet —L. Eye. ♂ 1 Facet —L. Eye.	52	14	9	3	1	4	1	0	0	38%
III	Stock 31. ♀ 1 Facet —R. Eye. ♂ 2 Facets—R. Eye.	39	3	4	0	1	1	1	0	0	20%
III	Stock 32. ♀ 1 Facet —R. Eye. ♂ 1 Facet —R. Eye.	11	0	3	1	1	2	1	0	0	42%
III	Stock 33. ♀ No Pig. Facets. ♂ No Pig. Facets.	26	2	4	1	2	1	1	0	0	30%
III	Stock 34. ♀ 1 Facet —R. Eye. ♂ 1 Facet —R. Eye.	53	5	10	3	5	2	0	0	0	32%
III	Stock 35. ♀ 3 Facets—R. Eye. ♂ 2 Facets—R. Eye.	12	1	5	1	4	0	0	0	0	48%
III	Stock 36. ♀ 2 Facets—L. Eye. ♂ 2 Facets—L. Eye.	7	1	1	0	0	0	0	0	0	22%
III	Stock 38. ♀ 2 Facets—L. Eye. ♂ 1 Facet —L. Eye.	7	3	2	0	0	0	1	0	0	46%
III	Stock 39. ♀ 2 R.Eye—1 L.Eye. ♂ 1 Facet —L. Eye.	63	11	12	0	0	3	8	0	0	35%
III	Stock 40. ♀ 1 Facet —L. Eye. ♂ Many F.—Both E.	46	3	3	0	1	4	0	0	0	19%
III	Stock 41. ♀ 1 Facet —L. Eye. ♂ 1 R.Eye—1 L.Eye.	19	10	1	5	1	3	1	0	0	52%
III	Stock 42. ♀ 1 Facet —R. Eye. ♂ 1 Facet —R. Eye.	34	13	9	2	2	5	7	0	0	53%
III	Stock 43. ♀ 1 Facet —R. Eye. ♂ 1 Facet —R. Eye.	16	4	4	4	1	2	2	0	0	51%
III	Stock 45. ♀ 1 Facet —L. Eye. ♂ 1 Facet —L. Eye.	39	10	10	1	1	2	2	1	0	41%
III	Totals of Gen. III.	424	80	77	21	20	29	25	1	0	37%
IV	Totals of 3 Cultures	81	5	7	1	2	1	1	0	0	17%

thirteen produced offspring. In all 677 flies were examined from these matings, of which 37 per cent. showed pigmented facets. The highest proportion for a single mating was 53 per cent. and the lowest 19 per cent. Three fourth generation matings were made and of the 98 flies which resulted 17 per cent. showed pigmented facets. A mass culture was made up from flies of this generation and kept in the laboratory for two months. When examined at the end of a month an occasional pigmented facet was to be found and at the end of two months on examining a large number of these flies no pigmented facets were found. The table below gives a summary of the results.

DISCUSSION

It is obvious that this pigmentation of individual facets does not behave as a simple Mendelian recessive, unless environmental or genetic modifying factors mask the visible effect of the factor in many flies, for when matings of flies both of which had the character were made in no case did all the flies show the character and generally less than 50 per cent. of them had pigmented facets.

Morgan (2) describes several flies which have appeared in his stocks in which some of the ommatidia were red and the rest white. One of the individuals which he mentions had about a fourth of one eye red and the rest white. He ascribes this condition to somatic mutation and this would seem to be the most plausible explanation for such cases; however, the large number of flies with pigmented facets, the fact that in most instances only one facet was affected, and the discontinuous distribution of the pigment where more than one facet was colored would seem to rule out somatic mutation as an explanation in the case under consideration.

The recurrence of this character in four generations in a rather large percentage of the flies, while the character was not to be found in other laboratory stocks raised under the same conditions, would seem to show that it was due to a genetic factor or factors. The flies in the same stock without colored facets and the wide variation in the intensity of pigmentation in the facets affected seem to indicate the presence of modifying factors, such as have been shown to develop in stocks of other mutations. Possibly there were many normal overlaps carrying the factor but not showing the character. The small number of flies in many of the

cultures was due in part to the small culture vials and crowding of the larvae.

An examination of the appended table will show that the character behaves in much the same way as other asymmetrical characters, notably eyeless (3). A pair of flies in which pigmented facets occur only in the right eyes give offspring with all possible distribution of pigmented facets. Two flies occurred in which both eyes were mottled with many colored facets of varying intensities of pigmentation and one fly in which the left eye was so mottled. But from one of these flies no larger percentage of offspring showing the character was secured than from other matings.

Many mutations have arisen in Drosophila which have affected the pigmentation of the eye uniformly by dilution and in the case of white by the removal of all pigment. The present case seems to show that genetic factors may affect the distribution of pigment throughout the smaller units of the compound eye in somewhat the same way as genetic factors control the distribution of pigment in the coat patterns of many of the mammals.

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QUADRUPLE ALLELOMORPHS AFFECTING EYE-COLOR IN HABROBRACON

COMPOUND eyes and ocelli in the wild type of *Habrobracon juglandis* (Ashmead) are jet black in color. On March 11, 1920, a type female, isolated as a virgin, produced 253 black-eyed males and one male with orange eyes and ocelli. This male was crossed

to type females and the method of its inheritance studied (Whiting, P. W., 1921). Orange (o) was found to act as a simple recessive to type (O), eye color and ocellus color behaving as a single unit. No linkage appeared between this factor and that for defective venation (d), the main factor for defect of r_4, a vein in the primary wings. The mode of inheritance is sex-linkoid, since in this form males are produced by haploid parthenogenesis, females from fertilized eggs. In all orange stocks derived from this mutant male the eyes vary in color from very light cream to a deep red and from yellowish orange to pink. No cause for this variation has been found.

On June 24, 1924, an orange female mated to orange male, probably defective, produced in the first bottle six orange males, five orange defective males, fourteen orange females and eight orange defective females; in the second bottle seven orange males, five orange defective males, six orange females, three orange defective females and four males, two normal and two defective, with eyes and ocelli of a color decidedly different from any variation of the orange character. It was of a definite greenish tinge; a greenish white describes it best, although in some lights it appeared slightly pinkish. The term "ivory" (o^i) has been used for it in absence of a better descriptive word. It implies perhaps too much of a cream white. Two of the mutant males were dead when found, the third was very weak, while the fourth was rather dried out but still able to feed on honey water. The mother had been discarded before the mutants were found. She was of mixed ancestry, being made up of about 21 per cent. Iowa City stock and 79 per cent. Lancaster, the latter introducing d, the main factor for defective venation. The Iowa City stock did not have this factor. The ivory mutants were found by P. W. Whiting and the following tests of ivory with orange were made by him.

One mating of the surviving mutant male was secured. He had normal venation and was crossed to an orange virgin female from stock 10, a stock carrying the main factor (d) for defective venation. From this mating there were produced seventeen orange males, 142 orange defective males, seventeen orange females and fifteen orange defective females. The males would carry a single dose of d and o, since they inherit from the mother alone, while the females have inherited from both parents and have Dd and at least one dose of o. Since D is almost completely dominant over d, the F_1 females would be expected to have nor-

mal venation for the most part. The fact that about 47 per cent. of them are defective would indicate that a modifying factor has been brought in which tends to make Dd females appear defective.

Six sisters of the ivory mutants were tested but produced only orange sons. Five F_1 orange females with normal venation from the cross of orange defective stock 10 female by the ivory mutant male were tested and produced 104 orange males, ninety-eight orange defective males, 107 ivory males, 103 ivory defective males, sixty-six orange females and sixty-nine orange defective females. Seven F_1 orange females with defective venation were likewise tested and produced 243 orange males, 199 orange defective males, 244 ivory males, 224 ivory defective males, fifty orange females, and sixty-two orange defective females. From these data it can be observed that orange is dominant to ivory and that orange and ivory segregate into a one to one ratio in F_2; also that the factor d shows free segregation and is therefore probably not in the same chromosome as the orange locus with which it entered the cross. A comparison of the results from the normal and defective F_1 females indicates that genetically they are the same.

Ivory-eyed high defective females were chosen from subsequent cultures and from these the highly defective ivory stock 17 was derived.

In September, 1924, the problem of testing the relationship of ivory to type eye color was turned over to the junior author. Stock 1, a black-eyed stock, was used in most of the crosses. Twenty-two crosses of stock 1 females by stock 17 males were made. Since the mothers were black, all the sons should be black, while the eye color of the daughters would depend upon dominance. From these crosses 338 black males and 272 black females were produced, the latter result indicating that black is dominant to ivory. Forty-two of the F_1 females from this type of mating were tested, thirty-three of which had mated with black-eyed brothers and nine of which were virgin. The mated females produced in all 390 black males, 310 ivory males and 376 black females, half of which should be heterozygous for ivory. The nine unmated females produced 133 black males and 139 ivory males. From these data we see that ivory and black segregate in the F_2 generation from heterozygous females.

Seventeen of the reciprocal crosses were also made, that is, stock 17 females by stock 1 males. The F_1 males should here be

ivory like their mothers, except in a few cases of patrocliny, where males arise from fertilized eggs and are therefore black-eyed like the F_1 females. From the seventeen matings there were produced nineteen black "patroclinous" males, 274 ivory males and 198 black females. Thirty-one F_1 females were bred out, twelve as virgin. The nineteen females mated to ivory brothers produced 209 black males, 243 ivory males, 110 black females, 129 ivory females. The twelve F_1 virgin females produced 286 black males and 320 ivory males. Total male offspring of all F_1 females number 1,018 type and 1,012 ivory.

Nine matings of F_2 and F_3 heterozygous females showed the same segregation, producing 125 black males and 133 ivory males.

Four crosses were made involving stock 17 females and males from stock 11, a black-eyed stock very different from stock 1 in ancestry. These resulted in twenty-two ivory males and thirty-three black females. Ten of the F_1 black females, mated to ivory brothers, produced 103 black males, eighty-two ivory males, 236 black females and 233 ivory females.

When the ivory-eyed males were found it was thought that they might have resulted from a mutation in a modifier of orange rather than from a change in the orange locus itself. If this is the case the orange locus and that of the modifier must be very close together, for no crossovers resulting in a reconstitution of orange or a modification of black have occurred among the large numbers of wasps counted. The ivory mutant acts consistently like a third allelomorph to orange and black.

In both the orange-eyed and ivory-eyed mutants the ocelli changed in color along with the compound eyes, and have, as far as noted, with the exception of a single eye-mosaic male, always been of the same color. In January, 1925, from a cross of stock 17 female by stock 1 male made by the senior author there appeared 35 of the expected black-eyed daughters in addition to one female with black compound eyes and light ocelli. It was thought at first that this was a mosaic and that the region in which the ocelli were located had been developed from cells of maternal origin only. She was set with caterpillars and produced six ivory males, twenty-one ivory females and seven males and fourteen females, at first described as black. Towards the end of the counts, more careful examination brought out the fact that the ocelli in these so-called blacks were considerably lighter than those of straight black stocks, although not as light as those

of the orange or ivory stocks. Two virgin daughters with light ocelli were tested, one producing but six males, all with light ocelli, the other thirty-one such males and thirty-eight ivory-eyed males. For the sake of brevity the individuals with light ocelli will be called "light" and the gene symbolized by o^l. One mated light daughter of the mutant produced twenty-eight light males, twenty-two ivory males and forty-seven light females. Five of these females, mated to light or ivory brothers, gave forty-five light males, twenty-nine ivory males, forty-six light females and twelve ivory females. One cross of ivory female by light male gave three light patroclinous males, fifty-six ivory males and twenty-five light females. Two F_1 light females, unmated, gave sixty-one light males and forty-seven ivory males. Summarizing all matings involving light females heterozygous for ivory we have four bred as virgin giving 121 light males and 105 ivory males; nine mated to ivory males giving eighty-nine light males, ninety-two ivory males, eighty-six light females, seventy-nine ivory females; five mated to light males giving eighty-seven light males, fifty-three ivory males and 142 light females. These eighteen heterozygous females produced in all 297 light males and 250 ivory males.

The matings indicate that light behaves as a unit and acts as an allelomorph to ivory. A pure breeding light stock (18) was derived after these crosses were made. The ocelli have a tendency to be lighter in the females carrying ivory or orange than in homozygous females. They are also lighter in the latter than in the males, so that it is sometimes difficult to distinguish between light and type males.

One homozygous light female was crossed to an orange male. She produced nineteen light males and forty-three light females. indicating that light is dominant to orange. Seven matings of orange female by light male resulted in nine patroclinous males, 243 orange males and 273 light females. Six unmated F_1 light females carrying orange produced 239 light males and 274 orange males. Fourteen such females crossed to orange brothers gave 202 light males, 242 orange males, 145 light females and 190 orange females. Two similar females crossed to light brothers gave nineteen light males, fourteen orange males and thirty-two light females. The twenty-two F_1 females, heterozygous for light and orange, gave in all 460 light males and 530 orange males. These data indicate that light is a dominant allelomorph to orange.

Five black-eyed females from stock 1 were mated to light males and produced all black offspring, seventy black males and 154 black females. Four of the black F_1 females bred as virgins gave 113 black males and 130 light males. One F_1 female crossed to light male gave eighteen black males, twenty-two light males, three black females and two light females. Four F_1 females crossed to black brothers gave fifty-seven black males, forty-six light males and 103 black females. Since black males and light males are rather difficult to distinguish at all times, only those were listed as light that had unquestionably light ocelli. The others were listed as black. The nine F_1 females heterozygous for black and light produced in all 188 black males to 198 light males.

From the data here presented it would seem that we have a series of allelomorphs, black (O), light (o^l), orange (o) and ivory (o^i), in decreasing order of dominance.

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A CRETACEOUS FISH SPINE

THE exact geographic and geologic data concerning this Cretaceous fish spine are uncertain. It came from somewhere in western Kansas. Its taxonomic position is quite vague. It may be a member of any of a number of large teleosts. It was pointed out to me as a portion of a fossil palm leaf, but the histological character is that of osteodentine. When I first noticed this ichthyodorulite it was serving, with other fossils, as an edging for a flower bed in my neighbor's yard. His father had been, in an amateur way, an observer and collector of natural objects which he treasured during his lifetime, but, since they meant nothing to others, were dumped in the side yard. Among the shells, corals and other marine findings were some of my Cretaceous friends, a plesiosaur and a mosasaur, pieces of fish and turtle bones; but none were so well preserved as this fragment of a fish spine. My eyes were attracted to it by the clean-cut manner in which the sub-divisions were exhibited. I placed the


1
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object in my pocket, with my friend's consent, remarking that it would make a good paper weight. Some years later when particularly interested in the minute anatomy of fossil objects, I had microscopical sections made from it, for purposes of comparison, with the results shown in the two figures.

It is not my intention here to point out the very many interesting histological features these two photomicrographs show but to call attention to the great number of calcite-filled cracks which have broken up the spine structure. This is especially evident near the center of the upper photomicrograph.

The calcite and other crystals, evident in this picture, form fully 30 per cent. of the substance of the spine at this particular level. The field shown in the lower figure is not so badly broken up but still greatly disturbed. This condition is quite common among certain types of fossil vertebrate remains, but is evident only on microscopic examination. I have found it abundant in the scores of slides of bone from other Cretaceous vertebrates, and especially so in the mosasaurs. Dinosaur bones are not so frequently fractured by the intruding crystals. There is enough, however, to make a considerable difference in the size and shape of the bone. While the Mesozoic fossils are highly vascular there is still a superabundance of mineral crystals which may make a great deal of difference in the bone.

I would like to suggest that this internal fractioning was characteristic of the buoyant, aquatic vertebrates, were it not for the interesting fact that the bones of many genera of Permian Amphibia show areas equally broken up in the interior of the bone, detectable only with a microscope. The bones which have medullary canals are not so readily affected by such internal fracturing, but often Paleozoic and Mesozoic bones have no well-developed medullary space. The nature of the matrix obviously has much to do with this condition, and some bones exhibit more crystalline content than others.

The interpretation of the form of fossil bones is seriously interfered with by the intrusion of post-fossilization crystals. It is customary for writers to list a number of carefully selected measurements of their fossils, but often they do not know that these measurements may indicate a magnification of as much as one third over what the bone measured in life. Their elaborate determinations of form may be entirely wrong because of the swollen condition of the part due to the inclusion of crystals

which disrupt the bone and increase its size. Without this caution, too, a bone may be regarded as diseased. I am not sure that the bone is greatly elongated, but the shaft is often swollen by the intruding crystals. The swelling of the part is often unequal, so we may be sure that our fossils from certain horizons, rich in calcium, only partially represent the true nature of the animal.

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A PACIFIC SALMON WITH THREE EYES[1]

The specimen to be described was presented to the writer by Dr. W. H. Rich, chief of the Division of Scientific Inquiry, U. S. Bureau of Fisheries, and was one of a collection of salmon embryos made at the Battle Creek, California, government hatchery.

The cause of the anomaly is not known, although of course we are aware that lack of oxygen, presence of radium in the water, low temperature and lack of light may induce abnormal development in trout and salmon.

The abnormal specimen was studied in comparison with two controls from the same lot of eggs and then sectioned and compared with a control.

The specimen was 2.8 cm in length and presented a most peculiar appearance (Figs. 1 and 2), since it possessed in addition to two lateral eyes, normally placed, a third eye situated just behind the normal left one. The normal eyes were about 2.5 mm in diameter, while the third eye was but 1.7 mm in its antero-posterior diameter. In addition to the normal snout an additional one was present (Fig. 2). In all other external respects the anomalous specimen was normal and it had apparently reached about the same size as its fellows, since they were 2.5 cm and 2.7 cm in length, respectively.

The specimen was embedded in paraffin, and sections 15 and 20 microns thick were stained with haemotoxylin and eosin and compared with similar sections from one of the normal fish. Photographs were made by means of the Euscope and the draw-

[1] Teratological Studies—X. From the Woods Hole Laboratory of the U. S. Bureau of Fisheries.

ings shown in Figs. 3, 4, 5 and 6 were traced from certain of the photographs.

In Fig. 3 the brain and eyes of the control salmon are shown, with the normal optic nerves proceeding from the retina to the thalamencephalon.

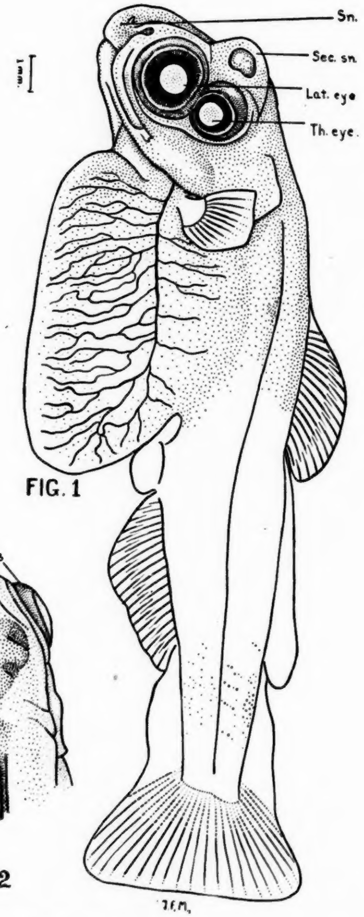

FIG. 1

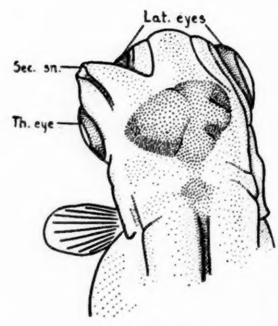

FIG. 2

FIG. 1. Lateral aspect of the three eyed salmon.

FIG. 2. Dorsal view of the head.

Sn., Snout; Sec. sn., Secondary snout; Lat. eye., Lateral eye; The. eye., Third eye.

In Fig. 4 the three eyes are shown, the well-developed accessory brain appearing but little smaller in the thalamic region than the original, while the third eye is sending its optic nerve in to attach to the thalamencephalon of the larger brain.

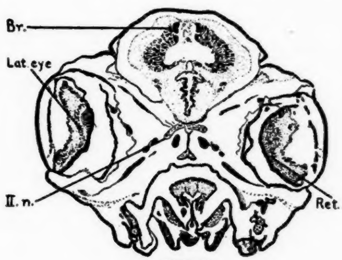

FIG. 3

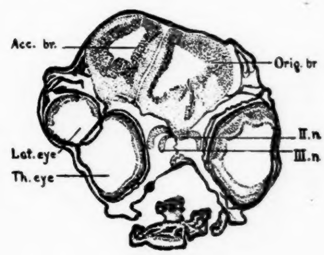

FIG. 4

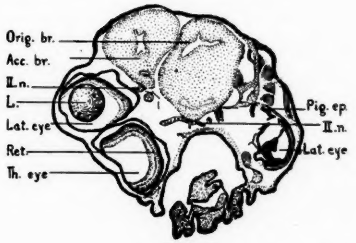

FIG. 5

JFD

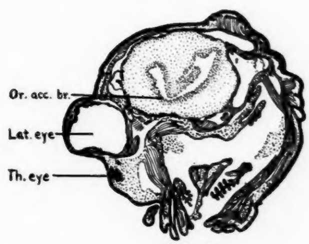

FIG. 6

FIG. 3. Section through the optic region of the control salmon.

FIGS. 4, 5, 6. Sections through the eyes and brains of the three eyed salmon.

Orig. br., Original brain; Acc. br., Accessory brain; Lat. eye., Lateral eye; Th. eye., Third eye; Or. acc. br., Point of origin of accessory lobe of brain; Ret., Retina; L., Lens; II. n., Optic nerve; III. n., Oculomotor nerve.

Fig. 5 illustrates again the relative sizes of the two brains and shows the optic nerve of the left lateral eye growing in to attach to the nearer thalamencephalon, which is of course that of the extra lobe.

In Fig. 6, taken from the region just anterior to the optic lobes, it is easy to see the origin of the cerebral division, that apparently had such a powerful influence on the nasal and optic outgrowth.

Anteriorly, it was discovered that a well-developed nasal outgrowth was not accompanied by a true mouth, although a pit appeared.

Posterior to the optic lobes there were no anomalies in the brain, and in fact no anomalies of the other systems were present.

Either the development of the left eye was retarded by the appearance of a new brain with its optic vesicle and snout or there has been a simple attachment of the ingrowing optic nerve to the most accessible portion of the central nervous system, the budded cerebrum.

The writer inclines to the latter view. It is quite evident that the accessory brain developed by fission of the primitive cerebrum, and it is also apparent that except for some inhibiting force, there might have been the development of a complete new head.

The specimen is apparently one step below the type of head described by the writer (1914), and by Gemmill (1903, 1912).

As regards the causes of such anomalies we have much speculation and considerable by way of facts. Temperature reduction or lack of oxygen or perhaps both were probably responsible for the condition indicated.

Summary

(1) A young salmon with three eyes and bilobed brain proved to have anomalous attachment of the optic nerves.

(2) The cause of the abnormality was probably lack of oxygen or low temperature.

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